Books are not absolutely dead things, but do contain a potency of life in them to be as active as that soul was whose progeny they are; nay, they do preserve as in a vial the purest efficacy and extraction of that living intellect that bred them

—John Milton

Prof RAMESHWAR SHARMA

Principal & Controller

Dedicated to the memory of a friend

MAURICE POWERS M D

who lost his life in the service of the
Royal Canadian Mounted Police

# Preface to the Second Edition

The preparation of a new edition gives an author an opportunity to re-crystallize and possibly purify a small precipitate of knowledge gathered on the filter paper of time Such an opportunity is appreciated

The purpose of the book remains the same that is to present those aspects of hematology which are significant to the medical technologist

The material is now divided into 7 chapters The first chapter deals with the collection and preparation of blood the next five chapters are concerned with some 27 blood tests and the final chapter considers the blood picture in various blood diseases

In the first edition the contents of the text was established by the simple process of asking students and technicians their thoughts and opinions These surveys showed that the consumers wanted a functional presentation of the subject matter used in their daily work In addition they wanted this material presented in a manner which was interesting clear and concise I endeavored to satisfy their wants

In preparing this edition I was guided by the criticism of reviewers and suggestions from another survey

I wish to thank the following reviewers for their criticism Dr C A Eick Dr Kurt Stern Dr Henry Kimpton Elsa S Kumke Bentley Class and Mrs Addine G Erskine I have endeavored to correct the mistakes and to make up the shortcomings mentioned in their reviews

I appreciate the suggestions of all those who answered the survey In particular I wish to thank the following Dr R L Northrip Dr Adelaide F Evenson Mr Bronnie A Corski Miss Clarice Sontag Dr Elias Cohen Mrs Dale

Pierson Anna R Persich Mrs Dell Pothier Portia M Frederick Mr Jay Lunnen and Mrs Hertz Seletzky I am especially grateful to Dr Elias Cohen who wrote detailed suggestions for the entire text

I also wish to acknowledge the help of the following Robert Pribbenow Gerald Henderson Valerie Mayfield Gertrude Denning Ann Brunner Nick Hoehn Ruthanne M Schupick Jim Kirk C Dean Hogarth and Irene Samolin

In conclusion suggestions and criticism from students technicians instructors and physicians would be appreciated

CHARLES F SHEPERD

GLENDALE ARIZONA

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# HEMATOLOGY FOR MEDICAL TECHNOLOGISTS

## Chapter 1

# Collection and Preparation of Blood

THIS book is concerned with the blood tests which are performed in the hematology section of a medical laboratory These tests are performed to combat disease restore health and lengthen life And the technician who performs these tests not only serves a noble cause but also plays a leading role in the drama of life death and disease

This chapter offers a brief discussion of the function and composition of blood and then presents the methods of obtaining and preparing blood for examination

### Function of Blood

The blood may be thought of as the body s most versatile servant It conveys the raw materials of protoplasm to countless millions of cells operating as factories to manufacture life itself The cells empty their waste products into its everflowing stream and this obliging servant carries them to the kidneys and other organs of elimination Blood aids in regulating the water content temperature and alkalinity of the tissues In time of danger it serves as a mechanized army transporting white cells and antibodies to battle infection and disease Quite often its factors of coagulation are called upon to help mend an abrasion laceration or incision It transfers hormones from their organs of production to their organs of consumption In order to discharge these duties this faithful servant works day and night traveling endless miles over the highways of an intricate system of arteries capillaries and veins

**Composition of the Blood**

The average man has about 5 quarts of blood. This can be separated into 2 quarts of cells and 3 quarts of plasma. The cells are solids and the plasma is a liquid.

The cells are classified as white cells, red cells, and platelets. In size, the white cells are largest, next in line are the red cells, and finally the platelets. In quantity, however, the red cells greatly predominate. For every 500 red cells there are approximately 30 platelets and only 1 white cell.

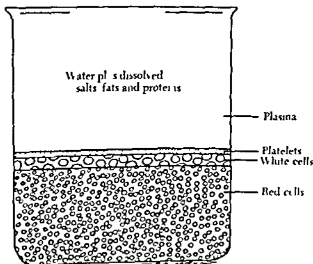



Fig. 1.—Composition of the Blood.

The plasma is made up of water and dissolved material, mainly salt, fats, and protein. The composition of the blood is illustrated in Figure 1.

## COLLECTION OF BLOOD

In order to perform some tests, only a few drops of blood are required. These few drops may be obtained by punc

turing a finger For other tests however larger amounts are needed and the blood must be obtained from a vein

This section discusses the methods of obtaining blood from a patient The material is presented as follows

Reassuring the Patient
Obtaining Blood from a Finger
Obtaining Blood from a Vein
Obtaining Blood from an Infant

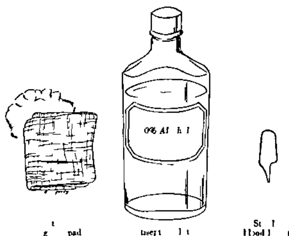


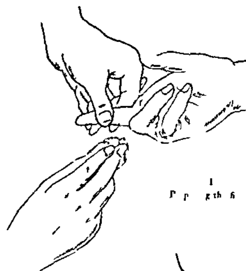

*Fig 2 —Materials needed for finger puncture*

**Reassuring the Patient**

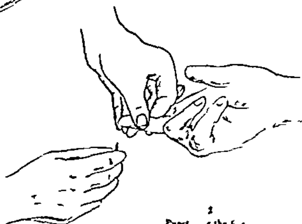
Reassuring the patient is the first step in obtaining a sample of blood With adults this may be accomplished by a pleasant smile an introductory remark or simply the stock statement This won t hurt very much Such thoughtful gestures take little time and are greatly appreciated by the patient The technician should avoid the attitude This is just another job I ve got to get the blood so brace yourself

With children extra care should be taken Endeavor to win the child s confidence Ask about his school games or

what he wants to be when he grows up Tell him that it won t hurt any more than being stuck with his mother s sewing needle A considerate technician usually has little difficulty and the display of thoughtfulness helps to condition the child for any future experiences On the other hand a thoughtless technician not only has trouble getting the blood but may cause the child to harbor a life-long fear of the puncture



1
P p g th fi



2
Punct g the f r

**Obtaining Blood from a Finger**

The materials needed for a finger puncture are a cotton or gauze pad an antiseptic solution and a sterile blood lancet (Fig 2)

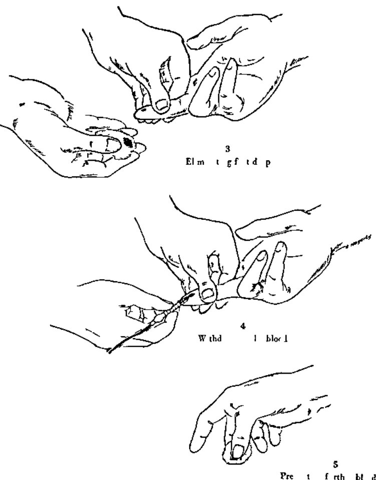



FIG 3 —Steps in the finger puncture

The finger is cleansed with the antiseptic solution an incision is made with the sterile blood lancet and drops of blood are available for examination A detailed explanation of each step follows

1 *Preparing the Finger*

The finger is cleansed with a gauze pad which has been moistened with 70 per cent alcohol or some similar antiseptic It is then dried so that the blood will form a rounded drop

2 *Puncturing the Finger*

The tip of the finger is punctured with a sterile lancet The puncture is made by grasping the finger firmly and making a quick deliberate stab Since a deep puncture is not any more painful than a superficial puncture it is best to go deep enough the first time and thereby avoid puncturing the patient a second time

3 *Eliminating the First Drop*

The first drop of blood may contain tissue juices Also the first drop may be contaminated with extraneous material which have been clinging to the surface of the skin When blood contains tissue juices and foreign particles it is not a true representative sample of the patient's blood Therefore the first drop of blood is always wiped away

4 *Withdrawing the Blood*

Gentle pressure is applied if necessary and drops of blood are taken for the desired examination Heavy pressure should be avoided because it may cause the flow of tissue juices and dilute the blood

5 *Preventing Further Bleeding*

When the desired amount of blood has been obtained an antiseptic pad is placed on the puncture The patient is then instructed to apply pressure to the wound until bleeding has ceased

The above steps in the finger puncture are illustrated in Figure 3

*Summary of the Finger Puncture*

1 Prepare the finger
2 Puncture the finger
3 Eliminate the first drop
4 Withdraw the blood
5 Prevent further bleeding

**Obtaining Blood from a Vein**

When more than a few drops are required for examination the blood is obtained from the veins of the forearm wrist or ankle Because the veins of the forearm are larger they are usually chosen However injury or repeated venipunctures may cause them to be inaccessible because of scar tissue soreness or bandaging Such cases make it necessary to use the veins of the wrist or ankle

The materials needed for a venipuncture are shown in the blood tray which is illustrated in Figure 4

If the venipuncture is to be made on a patient in a hospital room or ward it is always a good idea to check the blood tray for the essential materials before leaving the laboratory

In order to make a venipuncture of the forearm the patient and technician situate themselves so that the patient is comfortable and the technician can work with freedom of action If the patient is not confined to bed he should be seated as this position is not only more comfortable but highly desirable in case of fainting *

A bed patient should be asked to move to the edge of the bed and if necessary the bed table or chair are moved so that the technician can work with ease The blood tray is placed where it is handy and yet not in danger of being upset by any unexpected movements of the patient While these adjustments are being made the patient is being psychologically prepared for the venipuncture in the manner already suggested

The technician then prepares the needle and syringe applies a tourniquet cleanses the skin inserts the needle into a

* Should the patient faint hold a bottle of smelling salts below his nose If smelling salts are not available a piece of cotton moistened with *dilute* ammonium hydroxide will serve the purpose

The finger is cleansed with the antiseptic solution an incision is made with the sterile blood lancet and drops of blood are available for examination A detailed explanation of each step follows

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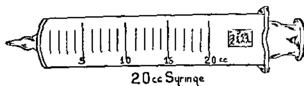
## 5 *Preventing Further Bleeding*

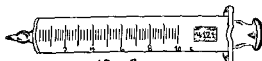
When the desired amount of blood has been obtained an antiseptic pad is placed on the puncture The patient is then instructed to apply pressure to the wound until bleeding has ceased

The above steps in the finger puncture are illustrated in Figure 3

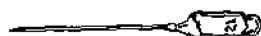
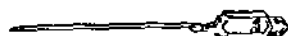
and 21 are commonly used for the veins of the forearm and gauge 25 is used for the veins of the wrist or ankle (Fig 5)

The choice of a syringe is governed by the amount of blood required A 10 cc syringe is most frequently used however if a large amount of blood is required a 20 cc syringe is employed (Fig 5)




| Gauge of Barrel | Length of Barrel |
|---|---|
| 21 | 1½ in |
| 21 | 1¼ in |
| 20 | 1½ in |
| 20 | 1¼ in |
| 25 | ¾ in |
| 25 | ½ in |

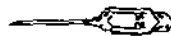



Fig 5 —Needles and syringes used for the venipuncture

When the needle and syringe have been chosen the needle is removed from its sterile container and firmly adjusted to the stem of the syringe—care being taken to keep the barrel of the needle sterile A check is made to see that the needle is not clogged by moving the plunger up and down and attempting to force air through the needle

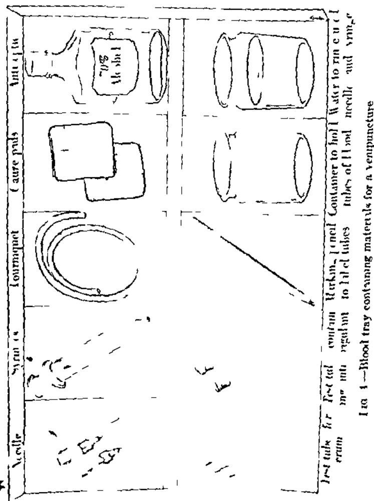



Fig. 4.—Blood tray containing materials for a venipuncture

vein and withdraws the blood. A detailed explanation of each step follows.

### Preparing the Needle and Syringe

The diameter of a needle is given by its gauge number; the smaller the number, the greater the diameter. Gauges 20

the vein A few cubic centimeters of air pumped into a vein could cause death

6 *Withdrawing the Blood*

When the needle enters the vein the plunger is pulled slowly back and the desired amount of blood is drawn into the syringe Should the blood start to enter the syringe and then cease the needle may have slipped out of the vein or gone through it If it has slipped out it should be carefully reinserted If it has gone through it should be slowly withdrawn until it is once again in the blood stream The touch which comes with experience is often the only means of telling whether the needle has slipped out or gone through the vein

If the vein is small and the blood is withdrawn too fast the removal of blood from the vein may exceed the flow of blood into the vein When this occurs blood can not be withdrawn and the vein is said to be collapsed Sometimes the situation can be remedied by twisting the needle slightly and allowing blood to pass into the collapsed vein

7 *Releasing the Tourniquet*

Before the needle is withdrawn the pressure must be released Otherwise blood would continue to flow from the hole made by the needle The pressure is removed by first instructing the patient to open his clenched fist and then releasing the tourniquet

8 *Withdrawing the Needle*

After the tourniquet is released the needle is withdrawn As the needle is withdrawn the antiseptic pad is applied to the puncture

9 *Preventing Bleeding*

In order to prevent bleeding the patient is instructed to apply pressure to the antiseptic pad covering the wound Sometimes the blood will seep into the subcutaneous tissue and leave a black and blue mark known as a hematoma

A hematoma may be caused by (a) failure to have the

Before use, all needles and syringes should be sterilized by one of the following methods: (a) autoclaving for 20 minutes at 15 pounds pressure, (b) dry heating in an oven at 170° C for 2 hours, or (c) boiling in water for 30 minutes and drying in an oven.

2. *Applying the Tourniquet*

It is desirable to enlarge the veins of the forearm so that they may become more prominent. This is accomplished by allowing blood to enter the arm by way of the arteries and blocking its return via the veins. A tourniquet (usually a piece of rubber tubing) placed above the bend in the elbow serves the purpose. The patient is instructed to clench his fist as this aids in building up the pressure.

3. *Selecting the Vein*

The most prominent vein is usually chosen. If the veins are not visible, opening and closing the fist may help to bring them out. Quite often the veins can not be seen, but may be felt. They will then reveal themselves as elastic tubes beneath the surface of the skin.

4. *Applying the Antiseptic*

At the proposed site of injection an antiseptic solution is applied with a piece of cotton or gauze pad.

5. *Inserting the Needle*

Many veins have a tendency to "roll over" when stuck with a needle and therefore must be held in position. Some technicians "fix" the vein by holding it with their thumb; others grasp the patient's arm just below the bend in the elbow and pull the skin taut. The needle is then held in line with the vein and inserted at approximately a 25 degree angle. From a mechanical point of view, it makes little difference whether the bevel of the needle faces up or down. However, most technicians prefer to have the bevel up since they feel better able to guide the passage of the needle. Precaution should always be taken against pumping air into

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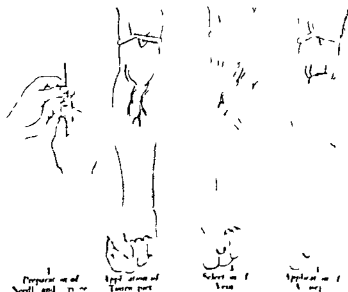
A hematoma may be caused by (*a*) failure to have the

needle completely in the vein (b) repeated punctures of the vein (c) failure to remove the tourniquet before withdrawing the needle and (d) failure to apply pressure to the wound for a sufficient length of time

The above steps in the venipuncture are illustrated in Figure 6

*Summary of the Venipuncture*

1 Prepare the needle and syringe
2 Apply the tourniquet
3 Select the vein
4 Apply the antiseptic
5 Insert the needle
6 Withdraw the blood
7 Release the tourniquet
8 Withdraw the needle
9 Prevent bleeding



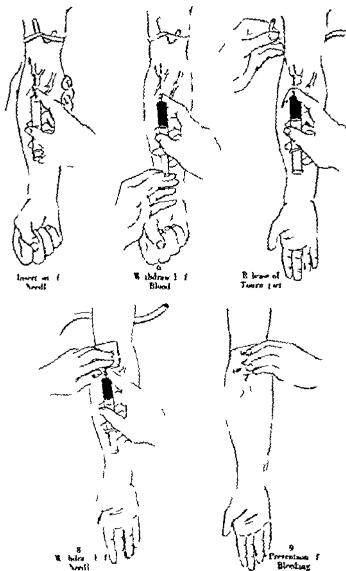


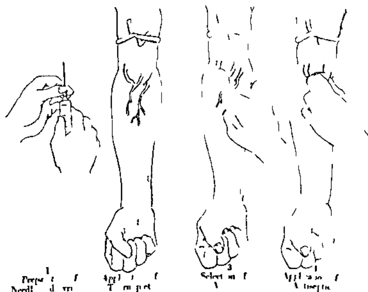

FIG 6 —Steps in the venipuncture

needle completely in the vein (*b*) repeated punctures of the vein (*c*) failure to remove the tourniquet before withdrawing the needle and (*d*) failure to apply pressure to the wound for a sufficient length of time

The above steps in the venipuncture are illustrated in Figure 6

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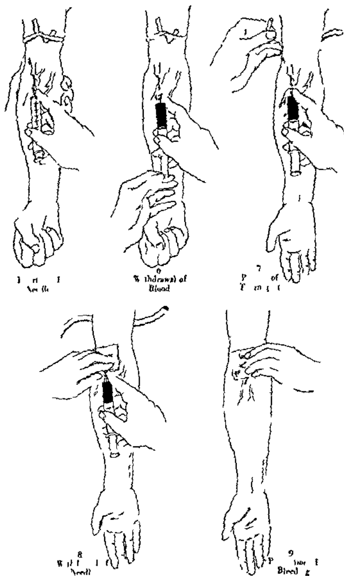
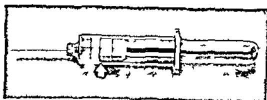


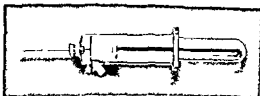

Fig 6 —Steps in the venipuncture

FIG 7 A to I —Collecting blood by means of a B D vacutainer (Courtesy of Becton Dickinson Company)

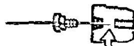




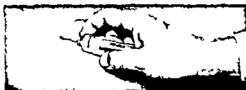
*A* SCREW NEEDLE INTO HOLDER AND PLACE TUBE IN HOLDER so needle point just touches stopper (See cross section)

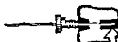






*B* PUSH TUBE FORWARD UNTIL TOP OF STOPPER MEETS GUIDE LINE then let go *Fi n if stopper re treats below guide line—leave in that po it on!* This embeds full point of the needle in stopper (see cro s section) thus avoiding blood l akage on entry into vein or premature lo s of vacuum

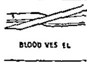




C WITH REAR POINT EMBEDDED IN STOPPER ENTER TISSUE—and immediately on tissue entry complete puncture of diaphragm (See cross section.)



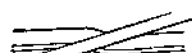




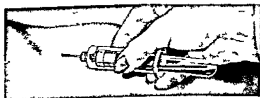

D IF IN VEIN—blood flows immediately. NOTE. *Technician with small hands* proceed as you would with a hypodermic syringe. Holder provides finger grip and tube acts as plunger (see inset). (*Continued* on pages 24-25)






*E* IF IN TISSUE instead of vein—blood will not be drawn Therefore continue until venipuncture is signalled by intake of blood into Vacutainer as shown in *D*

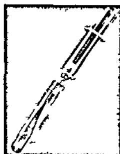


*F* WHERE VEIN CANNOT BE LOCATED—to conserve vacuum—release tube from rear cannula (see arrow) before withdrawing needle from tissue



*G* WHERE VENIPUNCTURE HAS BEEN MADE if *needle* is withdrawn *from* vein *just before* Vacutainer is completely filled the remaining vacuum will to a great degree cleanse the needle of blood thereby simplifying final cleaning





*H* To OBTAIN BLOOD DROPS gra p holder a illustrated and pre s bottom of tube

*I* To SPLIT BLOOD SAMPLES penetrate stopper of tube as illu trated

F IG (*Continued*) —Collecting blood by means of a B-D vacutainer (Courte y of Becton Dickinson and Company )

When blood is obtained by means of a venipuncture the needle should always be removed from the syringe before the blood is expelled into a test tube If the needle is not removed the sharp point of the needle causes the rupture or hemolysis of many red cells This hemolysis interferes in many tests

When the blood has been completely expelled the plunger of the syringe should be separated from the barrel If this is not done the syringe may become frozen when the blood dries up

*If the syringe does become frozen, it may be loosened by either (a) soaking in a 50-50 solution of alcohol and glycerin or (b) gently heating the barrel (causing it to expand) and then quickly pulling back the plunger before it also expands*

*Many technicians make it a practice to immediately wash a used syringe and needle with cold water Thus they remove the possibility of frozen syringes and clogged needles This policy is highly recommended*

*A new method of collecting blood is becoming popular in many hospital laboratories It consists of drawing blood into a tube by means of a vacuum The procedure is given in Figure 7*

**Obtaining Blood from an Infant**

If only a few drops of blood are needed for examination the blood may be obtained by puncturing the heel or big toe This is done in a manner similar to obtaining blood from a finger

If several cubic centimeters are required the blood may be obtained by making a venipucture of the scalp or neck This venipuncture however should be performed by a physician or experienced technician The procedure for obtaining blood from the external jugular vein is given below

1 Wrap the infant in a small sheet to prevent movement of arms and legs
2 Place the infant on the edge of a table or bed so that the head just hangs over the edge

3 Pin the sheet holding the baby to the table or bed
4 Turn the baby s head to one side and locate the external jugular vein If the baby cries —or can be induced to cry—the external jugular vein becomes quite prominent
5 Sterilize the area
6 Attach a sterile needle to a syringe
7 Hold the skin taut insert the needle and withdraw the blood
8 Withdraw the needle and place a piece of cot ton moistened with alcohol on the site of the puncture Apply gentle pressure until bleed ing has ceased
9 NOTE In obtaining blood from infants it is always a good policy to double check the wound and make certain that bleeding has ceased before leaving the ward or room

## PREPARATION OF BLOOD

For several determinations in hematology the technician *does not have to prepare the blood For the majority of* determinations however he must prepare the blood before the test can be performed For example in order to count the cells the technician dilutes the blood In order to study the different types of cells he makes a blood smear For other tests he may obtain plasma or serum

The preparations which are used in hematology are listed below and discussed on the following pages

1 Diluted blood
2 Blood smear
3 Oxalated blood
4 Blood plasma
5 Blood serum
6 Cell suspension

1 *Diluting the Blood*

Quite often the physician seeks information regarding the number of cells in the patient s blood This knowledge aids

him considerably in the diagnosis and treatment of many diseases The red cells white cells and platelets are so highly concentrated however, that the blood must be diluted in order to count them The dilution is made by sucking a measured portion of blood and diluting fluid into a pipet

## 2 *Making a Blood Smear*

The type maturity and abnormalities of both red and white cells furnish the physician with extremely significant information In order to study the cells a blood smear is made and the cells are stained The importance of making a good blood smear can not be overemphasized If it is poorly made the cells may be so distorted that it is impossible to recognize them This is particularly true in noting changes in the size shape and hemoglobin content of the red cells

The blood smear is made by placing a drop of blood on a glass slide and spreading it out so that the cells are evenly distributed The technique is illustrated in Figure 31 on page 80

## 3 *Preventing the Blood from Clotting*

The exact mechanism of blood coagulation is unknown However the theory of Howell offers a workable hypothesis According to this theory the blood plasma contains four substances which are involved in blood coagulation These substances are antiprothrombin prothrombin calcium and fibrinogen

Antiprothrombin inactivates prothrombin and prevents the circulating blood from clotting The blood will clot however if antiprothrombin can be neutralized When the skin is broken a substance called thromboplastin which is contained in the tissue juices neutralizes the antiprothrombin and allows the blood to clot

In many cases the physician is interested in the volume occupied by the red cells and the speed of their fall Since clotted blood can not be used to perform these tests an agent is needed to prevent the blood from clotting Such an agent is called an anticoagulant

An anticoagulant prevents the blood from clotting by

either removing or inactivating one of the necessary substances in blood coagulation For example calcium is used in the coagulation of blood Oxalate salts remove the calcium by forming calcium oxalate

Some of the more common anticoagulants and the amount required per cubic centimeter of blood are given in Table 1

*Table 1 —Common Anticoagulants*

| *Anticoagulant* | *Amount per cc of Blood* |
|---|---|
| Mixture of ammonium oxalate and potassium oxalate | 2 milligrams |
| Sodium citrate | 10 milligrams |
| Heparin | 0 2 milligram |
| Sequestrene | 1 milligram |

The mixture of ammonium oxalate and potassium oxalate is employed by many laboratories Its method of preparation is given in the Appendix page 238 When this anticoagulant is used the test tube containing the powdered oxalate is commonly referred to as an oxalated tube and when blood is added it is called oxalated blood

In order to obtain oxalated blood place about 5 cc of blood in an oxalated tube put the cork in the mouth of the tube and invert 8 to 10 times to dissolve the oxalate

The question is frequently asked How long can oxalated blood sit before a test must be set up? As a general rule oxalated blood may be preserved for about 4 hours by storing in the refrigerator Upon removal from the refrigerator it should be thoroughly mixed by inverting the tube 10 to 15 times

4 *Obtaining the Plasma*

A centrifuge is an instrument which spins fluids at a high rate of speed This forces the solid material to the bottom and leaves the liquid portion above A typical centrifuge with the operating directions is illustrated in Figure 8

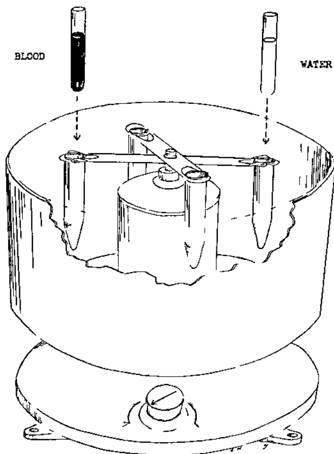



FIG 8 —Operation of Centrifuge (1) Place the test tube containing the blood in one holder (2) Place a test tube containing an *equal* amount of water in the *opposite* holder (This balances the centrifuge ) (3) Turn the operating dial a far as it will go

When oxalated blood is centrifuged the cells go to the bottom of the test tube The liquid portion above the cells is blood plasma This is used to determine the prothrombin time a test which studies the speed of blood coagulation

5 *Separating the Serum*

When a patient is to be given a transfusion his blood is tested for compatibility with the donor s blood The test is called a cross match and requires a sample of the patient s serum

The serum for the cross match is obtained by placing about 5 cc of blood in a test tube allowing it to clot and then centrifuging The fluid above the blood clot is serum It differs from plasma in that it contains no fibrinogen—a substance which has been used in the formation of the blood clot

6 *Making a Cell Suspension*

A cell suspension is used in typing and cross matching blood It is prepared by placing 2 drops of blood in about 6 cc of normal saline (preparation given below *) and inverting several times in order to mix the cells

Normal saline prevents the red cells from swelling or shrinking that is water neither enters nor leaves the cells Such a solution is said to be isotonic A salt solution which is less concentrated than normal saline is called hypotonic whereas a more concentrated solution is referred to as hypertonic

If the red cells were placed in a hypotonic solution water would pass through the cell membrane and enter the cells This would cause them to swell On the other hand if the cells were put in a hypertonic solution water would leave the cells and cause them to shrink

The student will recall from his previous studies that the above is simply an illustration of the principle of osmosis When two liquids of different densities are separated by a semipermeable membrane (the membrane covering the cells) the flow of water is always toward the greater density

The six different preparations of blood which have been discussed above are illustrated in Plate I

* Preparation of normal saline solution Using the analytical balance weigh out 8 50 Gm of sodium chloride Place in a 1 liter volumetric flask and add distilled water to the 1 liter mark Mix

A summary of the tests performed in hematology and the blood preparation required is given in Table 2 Note that several of the tests require no blood preparation the blood being used directly as it comes from the patient

### *Table 2 —Summary of the Tests Performed in Hematology and the Blood Preparation Required*

| *List of Tests* | *Blood Preparation Required* |
|---|---|
| White cell count | Diluted blood |
| Red cell count | Diluted blood |
| Hemoglobin estimation | Diluted blood or whole blood |
| Differential white cell count | Blood smear |
| Examination of stained red cells | Blood smear |
| Sedimentation rate | Oxalated blood |
| Hematocrit reading | Oxalated blood |
| Sickle cell examination | None or wet preparation |
| Fragility test | None |
| Reticulocyte count | Blood smear |
| Bleeding time | None |
| Coagulation time | None |
| Clot retraction time | None |
| Prothrombin time | Blood plasma |
| Platelet count | Diluted blood or blood smear |
| Capillary resistance test | None |
| ABO grouping | Oxalated blood or cell suspension |
| Rh typing | Oxalated blood or whole blood |
| Cross match | Blood serum and cell suspension |
| Coombs test | Cell suspension or serum |
| Rh titer | Serum |
| L E cells | Blood clot |
| Eosinophil count | Diluted blood |
| Spinal cell count | None |
| Malaria parasites | Blood smear |
| Bone marrow smears | Blood smear |
| Heterophile antibody test | Serum |

II AIF I



PATIENT S
BLOOD

D a lood nto tte an te

Mak a loo sl de a ta n the cell

Pla a t 5 c o alated t be and shak

Place abo t 5 c a t the let clot d centrifuge

Pla ew d no m al ne

DILUTED BLOOD

BLOOD SLIDE

OXALATED BLOOD

ce t f g

BLOOD PLASMA

BLOOD SERUM

CELL SUSPENSION

I NEP TI N THE B I I Ex I TI N

## Chapter 2

# Complete Blood Count

THE complete blood count is a routine determination which is made on all surgical and medical patients. It furnishes the physician with information which is extremely significant in the diagnosis and treatment of many diseases. Since more than half the requisitions coming into the hematology department call for a complete blood count and questions concerning the procedure are frequently found on student examinations, this chapter should be thoroughly mastered.

The complete blood count is commonly referred to as a CBC. It consists of the tests listed below:

- White cell count
- Red cell count
- Hemoglobin estimation
- Differential white cell count
- Examination of the stained red cells

These tests are discussed in detail on the following pages. A sample CBC report including the normal values is given in Figure 9.

## WHITE CELL COUNT

### Information Significant to the Student

The white cell count is the number of white cells in 1 cubic millimeter of blood. In health there are 5,000 to 10,000 cells per cubic millimeter. In some diseases the count rises; in other diseases the count falls. Quite often the rise and fall of the count serves as a barometer to indicate the course of a disease or the progress of infection. Thus the white cell count becomes a reliable spoke in the wheel of diagnosis and the physician turns to the technician for information regarding its deviations from normal values.

| Test | Patient's Values | Normal Values |
|---|---|---|
| *White Cell Count* | 6 800 | 5 000 to 10 000 per cu mm |
| *Red Cell Count* | 4 5 million | 4 0 to 5 5 million per cu mm |
| *Hemoglobin Estimation* | 13 grams (84%) | 12 to 17 grams (78 to 110%) |
| *Differential White Cell Count* | | |
| Neutrophilic myelocytes | 0 | 0% |
| Neutrophilic metamyelocytes | 0 | 0% |
| Neutrophilic band cells | 3 | 2 to 6% |
| Neutrophilic segmented cells | 60 | 55 to 75% |
| Lymphocytes | 32 | 20 to 35% |
| Monocytes | 4 | 2 to 6% |
| Eosinophilic segmented cells | 1 | 1 to 3% |
| Basophilic segmented cells | 0 | 0 to 1% |
| Miscellaneous white cells (plasma cells L E cell etc ) | 0 | 0% |
| *Examination of the Stained Red Cells* | | |
| Hypochromia (slight mod marked) | 0 | absent |
| Anisocytosis | 0 | absent |
| Poikilocytosis | 0 | absent |
| Miscellaneous red cells (nucleated red cells etc ) | 0 | absent |

Fig 9 —Sample CBC report

An increase above the normal values is called a leukocytosis whereas a drop below the normal is referred to as a leukopenia Some diseases where these abnormalities may be expected are listed below

| *High white cell count (leukocytosis)* | *Low white cell count (leukopenia)* |
|---|---|
| leukemia | measles |
| pneumonia | influenza |
| diphtheria | brucellosis |
| meningitis | typhoid fever |
| appendicitis | malignant neutropenia |

**Procedure for the White Cell Count**

It is strongly urged that the student first read this entire procedure through and then perform a white cell count by following the summary on page 47

The blood for the white cell count is obtained from a finger puncture or venipuncture The procedure for the count consists of four steps They will be considered in detail and discussed in the following order

1 Diluting the blood
2 Charging the counting chamber
3 Counting the cells
4 Making the calculations

1 *Diluting* the *Blood*

The dilution of the blood for the white cell count serves a dual purpose First it facilitates the counting process by *suspending and dispersing the white cells Second* it dissolves the red cells This is essential since the red cells greatly outnumber the white cells and would therefore interfere in the count

The blood is diluted with a weak solution of hydrochloric or acetic acid The diluting fluids are often referred to as WBC diluting fluids the WBC standing for white blood count The preparation of the diluting fluids is given below *

The dilution is made with a white cell pipet which is illustrated in Figure 10 The stem of the pipet is the portion from 0 0 to 1 0 and the mixing chamber is the bulb-like portion from 1 0 to 11 0 The volume of the stem is exactly ten times less than the volume of the mixing chamber Thus we can say that *the stem holds 1 unit of volume and the mixing chamber holds 10 units of volume*

Preparation of diluting fluid

*a* 1% (approximate 0 1 normal) hydrochloric acid Place 99 cc of distilled water in a container Add 1 cc of concentrated hydrochloric acid Mix

*b* 2% acetic acid Place 98 cc of distilled water in a container Add 2 cc of glacial acetic acid Mix

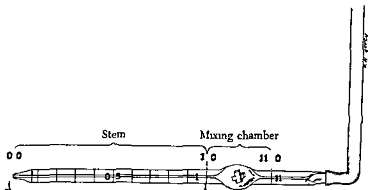



Fig 10 — White cell pipet attached to rubber sucking tube

Fig 11 — Diluting the blood (From Kolmer, Spaulding and Robinson, *Approved Laboratory Technic*, 5th edition, 1951. Courtesy of Appleton-Century-Crofts, Inc.)

To dilute the blood the technician attaches one end of a rubber sucking tube to the pipet and places the other end in his mouth He first sucks up blood then wipes the tip of the pipet with cotton and finally sucks up diluting fluid The sucking up process is controlled with the tip of the tongue Before detaching the rubber tube from the pipet he holds his finger over the bottom of the pipet to prevent the loss of fluid An illustration of a technician diluting the blood is given in Figure 11

In drawing up blood and diluting fluid the mixing chamber is filled with a solution of blood and diluting fluid The stem of the pipet still contains diluting fluid but this is discarded later on in the procedure

When blood is drawn to the 1 0 mark and diluting fluid to the 11 0 mark the 10 units of the mixing chamber contain 1 part blood and 9 parts diluting fluid Since this is 1 part blood in 10 parts of solution (blood plus diluting fluid) the blood has been diluted 1 in 10 The number 10 is known as the dilution factor

When blood is drawn to the 0 5 mark and diluting fluid to the 11 0 mark the 10 units of the mixing chamber contain 0 5 part blood and 9 5 parts diluting fluid Since this is 0 5 part blood in 10 0 parts of solution (blood plus diluting fluid) the blood has been diluted 1 in 20 And the number 20 is the dilution factor It should be noticed that the dilution was doubled by simply taking half the volume of blood used for the 1 in 10 dilution

The above dilutions usually give students some trouble A good way to remember the 1 in 20 dilution is to recall that it is somewhat similar to a nickel being one-twentieth part of a dollar Thus we may look upon the blood as a nickel the diluting fluid 95 cents and the entire solution one dollar

The student should note that the actual number of cubic centimeters contained in the pipet is unimportant It is the ratio of blood to diluting fluid which is significant For example 1 cc of blood diluted with 19 cc of diluting fluid is a dilution of 1 in 20 and the dilution factor is 20 Also 1 pint of blood diluted with 19 pints of diluting fluid is a dilution of 1 in 20 and the dilution factor is 20 Thus the exact quantity of diluted blood on hand is irrelevant The significant

point is the degree of dilution which is expressed by the dilution factor This will be used later on in making the calculations *

The 1 in 10 dilution is sometimes used when the hemo globin is very low The 1 in 20 dilution however is used routinely And this will be the dilution considered in our presentation of the white cell count

*Summary* To dilute the blood 1 in 20

*a* Draw blood *exactly* to the 0 5 mark wiping the tip of the pipet with a piece of cotton

*b* Draw WBC diluting fluid to the 11 0 mark

2 *Charging the Counting Chamber*

A representative sample of the solution in the mixing chamber is to be transferred to a counting chamber (Fig 12) Therefore two procedures should be considered First in order to insure a uniform distribution of cells the blood and diluting fluid must be well mixed Second since the diluting fluid in the stem of the pipet did not take part in the dilution of the blood it must be discarded These two steps should be carefully performed as illustrated below for they are often major sources of error

The blood and diluting fluid are mixed by shaking the pipet for 2 to 3 minutes The pipet is shaken so that it follows the curve of a quarter circle—the curve between 9 and 12 o clock This is accomplished by the simple wrist movement shown in Figure 13

The bead in the mixing chamber serves two functions First it aids in the mixing process Second it helps identify the pipet the white cell pipet having a white bead and the red cell pipet having a red bead

When the solution has been well mixed the diluting fluid in the stem of the pipet is discarded by allowing the first 4 drops to flow from the pipet This of course means that

* A table of commonly used weight and measures which explains cc and other volumes used in this chapter is given in the Appendix on page 236

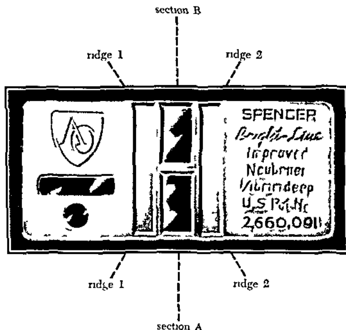



FIG 12 —Counting chamber The *longest* sides of a small coverglass are placed on the *raised* ridge 1 and 2 This makes a tiny distance between the coverglass and section *A* and section *B* These sections are used to count the cell Section *A* is usually used for the white cell count and section *B* for the red cell count

the solution in the mixing chamber will flow into the stem A *tiny* portion of this solution in the stem is then allowed to seep beneath the coverglass of the counting chamber The proper method of discarding the first 4 drops and charging the counting chamber is illustrated in Figure 14

The above mentioned figure also illustrates a properly charged counting chamber You will observe that only one side of the counting chamber is filled (the other side is for the red cell count) You will also note that the chamber is completely filled but not over filled or flooded This is most

point is the degree of dilution which is expressed by the dilution factor This will be used later on in making the calculations *

The 1 in 10 dilution is sometimes used when the hemoglobin is very low The 1 in 20 dilution however is used routinely And this will be the dilution considered in our presentation of the white cell count

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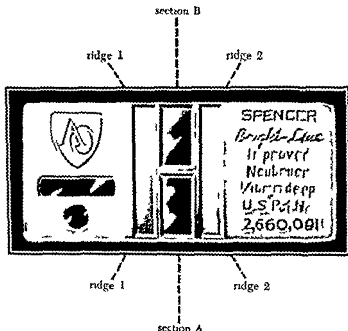



Fig 12 — Counting chamber The *longest* sides of a small coverglass are placed on the raised ridges 1 and 2 This makes a tiny distance between the coverglass and section A and section B These sections are used to count the cells Section *A* is usually used for the white cell count and section *B* for the red cell count

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important If you fail to fill the counting chamber in the proper manner simply wipe the chamber and coverglass with a soft cloth and try again

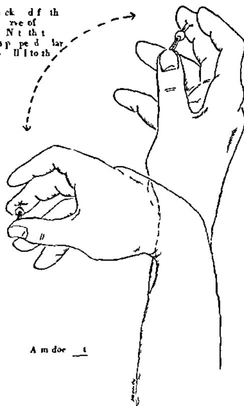



Fig 13 —Proper method of shaking a pipet

*Summary* To charge the counting chamber

a Shake the pipet for 2 to 3 minutes
b Discard the first 4 drops
c Transfer a *tiny* portion of the solution to the counting chamber

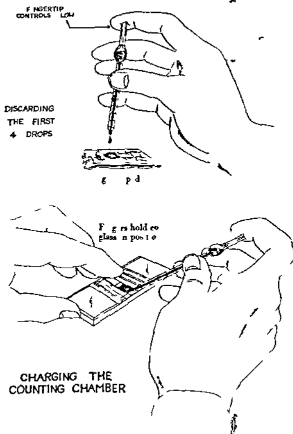



Fig 14 —Discarding the first 4 drops and charging the counting chamber

3 *Counting the Cells*

The count is made by means of a microscope which is illustrated in Figure 15 You will note that there are 3 magnifying devices or objectives These objectives are called the low power objective the high power objective and the oil immersion objective

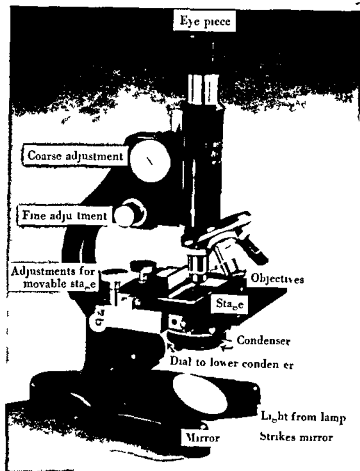



FIG 15 —Microscope

When the low power objective is used the cells are magnified 100 times This objective is used for the white cell count It is usually labelled 10× or 16 mm

When the high power objective is used the cells are magnified 450 times This objective is used for the red cell count It is usually labelled 45× or 4 mm

When the oil immersion objective is used the cells are magnified 1000 times This objective is used for the differential white cell count It is usually labelled 95X or *oil immersion* The name is derived from the fact that a drop of oil is put on the slide and the objective is then immersed in this oil

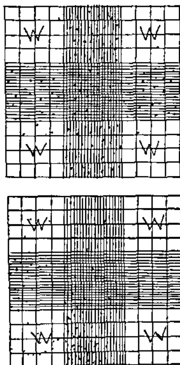



FIG 16 —Good and poor distribution of cells

In order to see the cells (1) The counting chamber is placed on the stage of the microscope (2) the low power (16 mm ) objective is switched into position over the counting chamber (3) the light is first centered up through the eye piece by adjusting the mirror (4) the light is then dimin

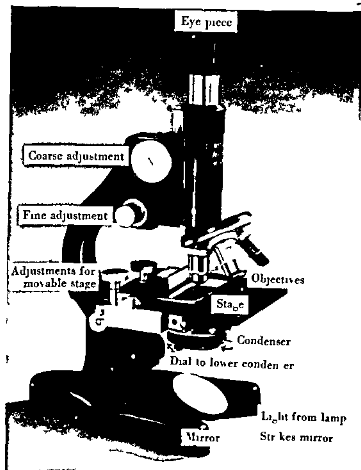



Fig. 15.—Microscope

When the low power objective is used the cells are magnified 100 times. This objective is used for the white cell count. It is usually labelled 10X or 16 mm.

When the high power objective is used the cells are magnified 450 times. This objective is used for the red cell count. It is usually labelled 45X or 4 mm.

Area of 1W section = 1 sq mm (for counting white cells)
Area of 1R section = 0.04 sq mm (for counting red cells)
Note 1R section is composed of 16 small squares each small square being 0.0025 sq mm.

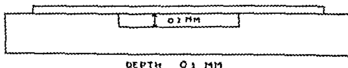



Fig 1 —The area and depth of the counting chamber

ished by lowering the condenser and (5) the cells are brought into focus by moving the coarse and fine adjustments.

By adjusting the movable stage of the microscope one of the "W" (white) sections in Figure 16 is brought into view. Each "W" section is made up of 16 small squares. One whole "W" section will occupy a single microscopic field.

A survey of the 4 "W" sections is then made to see if the cells are evenly distributed. If the cells are evenly distributed it indicates that the mixing process was satisfactory and that the diluting fluid in the stem of the pipet was discarded. However, if the distribution is poor, either or both of these steps were not satisfactorily completed. In such cases, the counting chamber should be wiped clean, the mixing process repeated, and the chamber charged again. Good and poor distribution of cells is illustrated in Figure 16.

As indicated in Figure 17, each "W" section has an area of 1.0 square millimeter and a depth of 0.1 millimeter. Its volume is found as follows:

| area | × depth | = volume |
|---|---|---|
| 1 sq mm | × 0.1 mm | = 0.1 cu mm |

Thus the volume of each "W" section is 0.1 cubic millimeter. The total volume of the four "W" sections is found as follows:

| Number of "W" sect | × Vol of 1 "W" sect | = Total vol of 4 "W" sect |
|---|---|---|
| 4 | × 0.1 cu mm | = 0.4 cu mm |

Thus the total volume of the four "W" sections is 0.4 cubic millimeter. When the cells in the four "W" sections are counted and added, the total is the number of cells in 0.4 cubic millimeter of solution.

*Summary.* To count the cells:

*a.* Bring the cells into focus with the low power (16 mm.) objective.

*b.* Check the distribution of the cells.

*c.* Count the cells in each of the four "W" sections.

*d.* Add the four counts.

*Summary* To make the calculations

*a* Multiply the total number of cells in the four W sections by 50

*Summary of the Procedure for the White Cell Count*

After reading the foregoing discussion perform a white cell count by following the steps in the summary below If you have trouble with any of the steps simply refer back to the detailed discussion It is to be expected that mistakes will be made Therefore do not get discouraged simply practice until you master the technique

1 Dilute the blood 1 in 20
   *a* Draw blood *exactly* to the 0 5 mark wiping the tip of the pipet with a piece of cotton
   *b* Draw WBC diluting fluid to the 11 0 mark
2 Charge the counting chamber
   *a* Shake the pipet for 2 to 3 minutes
   *b* Discard the first 4 drops
   *c* Transfer a *tiny* portion of the solution to the counting chamber
3 Count the Cells
   *a* Bring the cells into focus with the low power (16 mm ) objective
   *b* Check the distribution of the cells
   *c* Count the cells in each of the four W sections
   *d* Add the four counts
4 Make the Calculations
   *a* Multiply the total number of cells in the four W sections by 50

*Major Sources of Error in the White Cell Count*

1 Failure to draw blood *exactly* to the 0 5 mark
2 Failure to shake the pipet thoroughly
3 Failure to discard the first 4 drops
4 Failure to properly charge the counting chamber

### 4. *Making the Calculations*

The white cell count is the number of white cells in *1.0* cubic millimeter of *undiluted* blood. But we counted the number in *0.4* cubic millimeter of *diluted* blood! Therefore we must multiply the number counted by two correction factors.

The first correction factor compensates for the dilution of the blood. Since we diluted the blood 1 in 20, the dilution correction factor is 20. Of course if we had diluted the blood 1 in 10, the dilution correction factor would be 10.

The second correction factor compensates for the volume in which the cells were counted. Since we counted the cells in *0.4* cubic millimeter and not in *1.0* cubic millimeter, we must multiply by a volume correction factor. This is found as follows:

$$\text{Vol. cor. factor} = \frac{\text{volume desired}}{\text{volume used}} = \frac{1.0 \text{ cu. mm.}}{0.4 \text{ cu. mm.}} = 2.5$$

When the total number of cells in the four W sections is multiplied by the above correction factors of 20 and 2.5, the number of cells in one cubic millimeter of blood is obtained. This is the white cell count.

To illustrate, if the total number of cells in the four W sections is 100, the calculation is made as follows:

| Number of cells in 0.4 cu. mm. (4 W sections) | | Dil. cor. factor | | Vol. cor. factor | | Number of cells in 1.0 cu. mm. |
|---|---|---|---|---|---|---|
| 100 | × | 20 | × | 2.5 | = | 5,000 |

Since the product of the dilution and volume correction factors is 50 (20 × 2.5 = 50), the calculation can be made by simply multiplying the number of cells in the four W sections by 50. Thus:

| Number of cells in 0.4 cu. mm. (4 W sections) | | Correction factors | | Number of cell in 1.0 cu. mm. |
|---|---|---|---|---|
| 100 | × | 50 | = | 5,000 |

# RED CELL COUNT

**Information Significant to the Student**

The red cell count is the number of red cells in one cubic millimeter of blood The normal values are 4 0 to 5 5 million cells per cubic millimeter A decrease in the count may be found in the anemias and leukemias An increase in the count may be seen in polycythemia vera and dehydration conditions Consequently when the physician is diagnosing or treating the above cases his inquiry is brought to focus on the red cell count

**Procedure for the Red Cell Count**

It is strongly recommended that the student first read this entire procedure through and then perform a red cell count by following the summary on page 54

The blood for the red cell count is obtained from a finger puncture or venipuncture The procedure follows the same general pattern as the white cell count It is broken down into the following four steps which will be discussed in detail

1 Diluting the blood
2 Charging the counting chamber
3 Counting the cells
4 Making the calculations

1 *Diluting the Blood*

The diluting fluid used for the red cell count is either Hayem s solution or Gower s solution These solutions are isotonic with the red cells and thereby prevent the cells from swelling or shrinking The diluting fluids are commonly re ferred to as RBC (red blood count) diluting fluids Their method of preparation is given in the Appendix

The red cell pipet which is used to make the dilution is illustrated in Figure 10 This pipet may be readily distin guished from the white cell pipet since it either has a red bead in the mixing chamber or a red stripe on the stem The stem of the pipet is the portion from 0 0 to 1 0 and the mixing chamber is the bulb-like portion from 1 0 to 101 The stem

*Cleaning the Counting Chamber and Pipets*

Immediately after use the counting chamber and cover glass should be cleaned with a soft cloth

The white cell pipet and red cell pipet are cleaned by means of a suction which is attached to a faucet The procedure follows

1 Attach the suction to a faucet as illustrated in Figure 18
2 Insert the pipet in the rubber tube as illustrated
3 Turn the faucet on full force
4 Allow the pipet to suck up first distilled water then alcohol and finally ether
5 NOTE Some technicians do not use the alcohol and ether They simply clean the pipets with distilled water and place them in a drying oven to dry This procedure is quite satisfactory and—less expensive

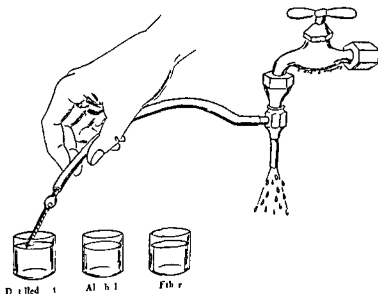


FIG 18 —Cleaning a pipet

*c* Transfer a *tiny* portion of the solution to the counting chamber

3 *Counting the Cells*

The counting chamber is first placed on the stage of the microscope By adjusting the mirror the light is centered up through the eye piece With the low power (16 mm) objective the central ruled area (the area containing the 5 R sections in Figure 17 page 45) is brought into view The light is then made suitable by moving the condenser up or down

The distribution of the cells is now checked If the distribution is poor the mixing process is repeated and the chamber is charged again However if the distribution is good the high power (4 mm) objective is switched into position over the counting chamber The cells should then be in focus If not they are brought into focus by using the *fine adjustment* of the microscope In this step the student should take particular precautions against moving the fine adjustment to the point where the objective touches or puts pressure upon the coverglass Such pressure could break the coverglass or even the counting chamber

One of the R sections illustrated in Figure 17 is then brought into view An R section is made up of 16 small squares Since the high power (4 mm) objective is being used one whole R section will occupy a single microscopic field

Each R section has an area of 0 04 square millimeter and a depth of 0 1 millimeter Its volume is found as follows

| | | | | |
|---|---|---|---|---|
| Area of 1 R section | × | Depth of 1 R section | = | Vol of 1 R section |
| 0 04 sq mm | × | 0 1 mm | = | 0 004 cu mm |

Thus each R section has a volume of 0 004 cubic millimeter The total volume of the five R sections is found as follows

| | | | | |
|---|---|---|---|---|
| Number of R sections | × | Vol of 1 R section | = | Total vol of 5 R sections |
| 5 | × | 0 004 cu mm | = | 0 02 cu mm |

contains 1 unit of volume and the mixing chamber holds 100 units of volume

When blood is drawn to the 0 5 mark and diluting fluid to the 101 mark the 100 units of the mixing chamber contain 0 5 units of blood and 99 5 units of diluting fluid Since this is 0 5 part blood in 100 parts of solution (blood plus diluting fluid) the blood has been diluted 1 in 200 The number 200 is the dilution factor

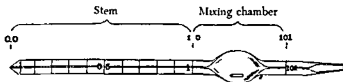



FIG 19 —Red cell pipet

*Summary* To dilute the blood 1 in 200

*a* Draw blood *exactly* to the 0 5 mark wiping the tip of the pipet with cotton

*b* Draw RBC diluting fluid to the 101 mark

2 *Charging the Counting Chamber*

The cells and diluting fluid are thoroughly mixed by shaking the pipet for 2 to 3 minutes Next the diluting fluid in the stem is discarded by letting the first 4 drops flow from the pipet A tiny portion of the solution is then transferred to the counting chamber and the cells are given a few minutes to settle *

*Summary* To charge the counting chamber

*a* Shake the pipet for 2 to 3 minutes

*b* Discard the first 4 drops

In a complete blood count if the white cell count is done first the red cells can be settling out while the white cells are being counted Doing the white cell count fir t has other advantages First when the low power objective is switched to the high power objective for the red cell count the cells can be brought into view without focusing the microscope Thus a step is saved Second coverslips are frequently broken by students trying to bring the field into focus with the high power objective Thus a coverslip may be saved

In order to obtain some measure of uniformity in counting cells which touch boundary lines of the R sections the cells touching *any* of the boundary lines on the left side and top of the R section are counted whereas cells touching *any* of the boundary lines on the right side and bottom of the R section are not counted This is illustrated in Figure 20

*Summary* To count the cells

*a* Bring the central portion of the ruled area into focus with the low power (16 mm ) objective

*b* Check the distribution of the cells

*c* Switch to the high power (4 mm ) objective

*d* Count the cells in the 5 R sections

*e* Add the five counts

4 *Making the Calculations*

The red cell count is the number of red cells in *1 0* cubic millimeter of *undiluted* blood But we counted the number in *0 02* cubic millimeter of *diluted* blood! Therefore we must multiply the number counted by two correction factors

The first correction factor compensates for the dilution of the blood Since we diluted the blood 1 in 200 the dilution correction factor is 200

The second correction factor compensates for the volume in which the cells were counted Since we counted the cells in *0 02* cubic millimeter and not in *1 0* cubic millimeter we must multiply by a volume correction factor This is found as follows

$$\text{Vol cor factor} = \frac{\text{volume desired}}{\text{volume used}} = \frac{1\ 00 \text{ cu mm}}{0\ 02 \text{ cu mm}} = 50$$

When the total number of cells in the 5 R sections is multiplied by the above correction factors of 200 and 50 the number of cells in 1 0 cubic millimeter of blood is obtained This is the red cell count

To illustrate if a total of 500 cells were counted in the 5 R sections the calculation is made as follows

Thus the total volume of the five "R" sections is 0.02 cubic millimeter.

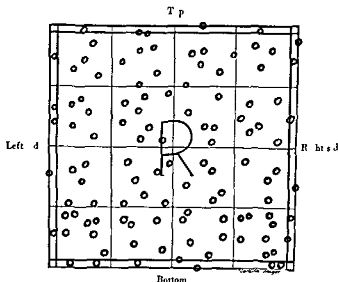



FIG 20.—Counting the Red Cells. Some counting chambers like the one shown above have 2 lines which serve as boundary lines for each "R" section. Other counting chambers however may have 3 lines. In counting cell, count the cells touching *any* of the boundary lines on the left side and top of the "R" section. Do not count the cells touching *any* of the boundary lines on the right side and bottom of the "R" section. In the above illustration the count by each small square is given below, the total count for the whole "R" section being 99.

$$
\begin{array}{ccccccc}
6 & \rightarrow & 7 & \rightarrow & 7 & \rightarrow & 4 \\
 & & & & & & \downarrow \\
7 & \leftarrow & 8 & \leftarrow & 5 & \leftarrow & 6 \\
\downarrow & & & & & & \\
6 & \rightarrow & 7 & \rightarrow & 5 & \rightarrow & 4 \\
 & & & & & & \downarrow \\
8 & \leftarrow & 5 & \leftarrow & 6 & \leftarrow & 8
\end{array}
$$

When the cells in the five "R" sections are counted and added, the total is the number of cells in 0.02 cubic millimeter of solution. White cells are often mistaken for red cells but their number is so few that they do not interfere in the count.

c Switch to the high power (4 mm) objective
d Count the cells in the five R sections
e Add the five counts

4 Make the calculations
a Multiply the total number of cells in the five R sections by 10 000

*Major Sources of Error in the Red Cell Count*

1 Failure to draw blood *exactly* to the 0 5 mark
2 Failure to shake the pipet thoroughly
3 Failure to discard the first 4 drops
4 Failure to properly charge the counting chamber

| Number of cells in 0 02 cu mm (5 R sections) | | Dil cor factor | | Vol cor factor | | Number of cells in 1 0 cu mm |
|---|---|---|---|---|---|---|
| 500 | × | 200 | × | 50 | = | 5 000 000 |

Since the product of the dilution and volume correction factors is 10 000 (200 × 50 = 10 000) the calculation can be made by simply multiplying the total number of cells in the five R sections by 10 000 Thus

| Number of cells in 0 02 cu mm (5 R section ) | | Correction factors | | Number of cells in 1 cu mm |
|---|---|---|---|---|
| 500 | × | 10 000 | = | 5 000 000 |

*Summary* To make the calculations

*a* Multiply the total number of cells in the five R sections by 10 000

*Summary of the Procedure for the Red Cell Count*

After reading the foregoing discussion the student should perform a red cell count by following the steps in this summary *If he has trouble with any of the steps he should refer* back to the detailed discussion

1 Dilute the blood 1 in *200*
   - *a* Draw blood *exactly* to the 0 5 mark wiping the tip of the pipet with cotton
   - *b* Draw RBC diluting fluid to the 101 mark
2 Charge the counting chamber
   - *a* Shake the pipet for 2 to 3 minutes
   - *b* Discard the first 4 drops
   - *c* Transfer a *tiny* portion of the solution to the counting chamber
3 Count the cell
   - *a* Bring the central portion of the ruled area into focus with the low power (16 mm ) objective
   - *b* Check the distribution of the cells

compared with the normal value for the particular instrument being used and the report is given in per cent of this normal value

To illustrate if a person has a hemoglobin of 14 grams and the normal value for the hemoglobinometer is also 14 grams he has a hemoglobin of 100 per cent A person with a hemoglobin of 7 grams would thus have a hemoglobin of 50 per cent

Unfortunately different hemoglobinometers have different normal values One hemoglobinometer may designate 14 grams as the normal value and consider this equivalent to 100 per cent Another instrument may designate 16 grams as the normal value and consider this equivalent to 100 per cent Thus a person with 15 grams of hemoglobin would be over 100 per cent with the first instrument and under 100 per cent with the second instrument

It is apparent that reporting hemoglobin in per cent has led to considerable confusion Many authorities therefore stress the necessity of doing away with the per cent and reporting only the grams of hemoglobin However the habit of discussing hemoglobin in percentage is rather deeply entrenched in the medical profession Consequently in order to alleviate the situation whenever the hemoglobin is reported in percentage the number of grams it represents should also be given

**Procedures for the Hemoglobin Estimation**

The methods of determining hemoglobin may be divided into two groups visual methods and photoelectric methods

Some visual methods compare the blood directly with colored standards Other visual methods convert the hemoglobin to acid hematin and then compare it with colored standards

Some photoelectric methods determine the hemoglobin as oxyhemoglobin This method is based on the fact that oxyhemoglobin has a characteristic color The intensity of this color is a measure of the hemoglobin present Other photoelectric methods determine the hemoglobin as cyanmethemoglobin This method calls for the conversion of all the differ

# HEMOGLOBIN ESTIMATION

**Information Significant to the Student**

Hemoglobin is made up of 2 substances One substance is heme an organic compound of iron The other substance is globin one of the simple proteins Under normal conditions, *hemoglobin is contained only in the red cells* In certain diseases however it may be found in the blood plasma In such cases the condition is referred to as hemoglobinemia

Since hemoglobin forms a temporary union with oxygen it enables oxygen to be transported from the lungs to the tissues It is also instrumental in conveying carbon dioxide from the tissues to the lungs When hemoglobin is laden with oxygen on its way to the tissues it is called oxyhemoglobin On the return trip to the lungs it is known as reduced hemoglobin

The normal values for hemoglobin are 12 to 17 grams per 100 cubic centimeters of blood Decreased values are found in anemia and leukemia Increased values are found in polycythemia vera and dehydration conditions

There are several factors apart from disease however which affect the hemoglobin concentration These factor are age locality and sex

In infants for example it is not uncommon to find a hemoglobin of 17 grams This same figure although normal for infants would be considered extremely high for women

The locality in which a person lives may also affect the hemoglobin *For example people living in mountainous regions* such as Denver normally have a higher hemoglobin than other people

And then there is the factor of sex To illustrate a hemoglobin of 10 5 grams in a pregnant woman may go unnoticed But the same figure in an apparently healthy man might cause the physician to raise his eyebrows

There are many different instruments for measuring hemoglobin These instruments are called hemoglobinometers The various hemoglobinometers measure the hemoglobin in grams per 100 cc of blood The number of grams is then

compared with the normal value for the particular instrument being used and the report is given in per cent of this normal value

To illustrate if a person has a hemoglobin of 14 grams and the normal value for the hemoglobinometer is also 14 grams he has a hemoglobin of 100 per cent A person with a hemoglobin of 7 grams would thus have a hemoglobin of 50 per cent

Unfortunately different hemoglobinometers have different normal values One hemoglobinometer may designate 14 grams as the normal value and consider this equivalent to 100 per cent Another instrument may designate 16 grams as the normal value and consider this equivalent to 100 per cent Thus a person with 15 grams of hemoglobin would be over 100 per cent with the first instrument and under 100 per cent with the second instrument

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The methods of determining hemoglobin may be divided into two groups visual methods and photoelectric methods

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Some photoelectric methods determine the hemoglobin as oxyhemoglobin This method is based on the fact that oxyhemoglobin has a characteristic color The intensity of this color is a measure of the hemoglobin present Other photoelectric methods determine the hemoglobin as cyanmethemoglobin This method calls for the conversion of all the differ

ent forms of hemoglobin (except sulfhemoglobin) to cyan methemoglobin.

The procedures for the estimation of hemoglobin are listed below and given on the following pages. The student should first determine which of these procedures is being used in his particular laboratory. He should then make a superficial reading of all the procedures and a careful reading of the procedure which he will use.

1. Dare Method
2. Tallqvist Method
3. Spencer Method
4. Sahli Hellige Method
5. Haden Hausser Method
6. Leitz Photrometer Procedure
7. Hellige Clinicol Procedure
8. Bausch & Lomb Spectronic 20 Procedure
9. Photovolt Lumetron Procedure
10. Coleman Spectrophotometer Procedure

1 DARE METHOD

1 Get materials for a finger puncture and the Dare hemoglobinometer (Fig 21)

2 Make the finger puncture wipe away the first drop of blood and allow a drop to be drawn between the glass plates of the instrument

3 Press the button to light the bulb match the color of the blood with the color scale and take the reading

4 Report the method used the grams of hemoglobin per 100 cc of blood and the per cent

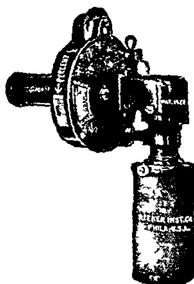



FIG 21 —Dare hemoglobinometer

2 TALLQVIST METHOD

1. Get materials for a finger puncture and the Tallqvist hemoglobin scale (Fig 22)
2. Make a finger puncture, wipe away the first drop of blood and place a drop on a piece of the absorbent paper which comes with the scale
3. Find the color on the scale which matches the color of the blood and take the reading
4. Report the method used, the grams of hemoglobin per 100 cc of blood and the per cent (see conversion table below)

*Table 3 —The Tallqvist Conversion Table*

| Gm | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| % | 6.3 | 12.6 | 18.9 | 25.2 | 31.5 | 37.8 | 44.1 | 50.4 | 56.7 |

| Gm | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|
| % | 63.0 | 69.3 | 75.6 | 81.9 | 88.2 | 94.5 | 100.8 | 107.1 | 113.4 |

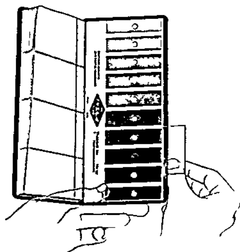


FIG 22 —Tallqvist hemoglobin scale

3 SPENCER METHOD

1 Get materials for a finger puncture and the Spencer hemoglobinometer (Fig 23)

2 Make a finger puncture wipe away the first drop of blood and place a drop on the open chamber of the hemoglobinometer Hemolyze the blood by stirring with the applicator and close the chamber

3 Press the illuminating switch look through the eyepiece match the colors and take the reading

4 Report the method used the grams of hemoglobin per 100 cc of blood and the per cent

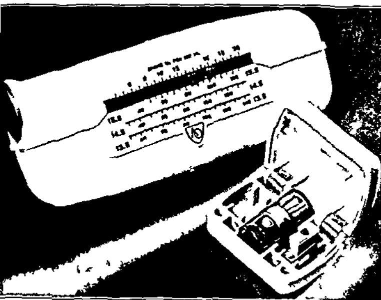


FIG 23 —Spencer hemoglobinometer

4 Sahli Hellige Method

1 Get materials for a finger puncture a bottle of 1% (approximately 0 1 normal) hydrochloric acid a pipet for measuring 20 cu mm of blood and the Sahli Hellige hemoglobinometer (Fig 24) Preparation of 1% hydrochloric acid is given in Appendix on page 240

2 Fill the graduated tube of the hemoglobinometer to the 10 mark with the 1% hydrochloric acid

3 Make a finger puncture wipe away the first drop and draw blood into the pipet to the 20 mark Wipe the tip of the pipet and expel the blood into the graduated tube Rinse the pipet several times by first drawing the solution up and then expelling it back into the graduated tube

4 *Add distilled water drop by drop mixing after each addition* until the color of the solution matches the color of the standard Take the reading corresponding to the height to which the solution has risen

5 Report the method used the grams of hemoglobin per 100 cc of blood and the per cent (see conversion table below)

***Table 4.—The Sahli Hellige Conversion Table***

| Gm | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| % | 6.5 | 13.0 | 19.5 | 26.0 | 32.5 | 39.0 | 45.5 | 51.9 | 58.4 |

| Gm | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|
| % | 64.9 | 71.4 | 77.9 | 84.4 | 90.9 | 97.4 | 103.9 | 110.4 | 116.9 |

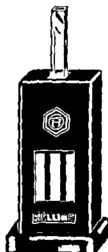



Fig. 24.—Sahli Hellige hemoglobinometer
(Courtesy of Hellige Inc.)

5 HADEN HAUSSER METHOD

1 Get materials for a finger puncture a white cell pipet and a bottle of 1% hydrochloric acid (preparation given in Appendix on page 240)

2 Make a finger puncture wipe away the first drop draw blood to the 0 5 mark of the pipet and the hydrochloric acid to the 11 0 mark (This is the same as the usual 1 in 20 dilution for the white cell count If the patient is anemic make a 1 in 10 dilution by drawing blood to the 1 0 mark and diluting fluid to the 11 0 mark The hemoglobin reading is then divided by 2 in order to compensate for this decreased dilution )

3 Discard the first 4 drops and fill the trough of the hemoglobinometer (Fig 25) Match the color of the blood with the scale and take the reading

4 Report the method used the grams of hemoglobin per 100 cc of blood and the percent (see conversion table below)

### *Table 5.—The Haden-Hausser Conversion Table*

| Gm | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| % | 6.5 | 13.0 | 19.5 | 26.0 | 32.5 | 39.0 | 45.5 | 51.9 | 58.4 |

| Gm | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|
| % | 64.9 | 71.4 | 77.9 | 84.4 | 90.9 | 97.4 | 103.9 | 110.4 | 116.9 |

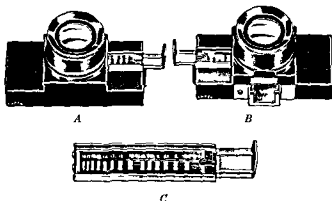


Fig. 25.—Haden-Hausser hemoglobinometer (clinical model). This instrument uses the same principle as the larger laboratory model but has no built-in light source. *A*, Front view showing comparator slide in position; *B*, rear view showing light filter in position; *C*, comparator slide with cover glass in position. (From Haden: Principles of Hematology.)

## 6 Leitz Photrometer Procedure (*Courtesy E Leitz Co*)

### *Hemoglobin*

Sheard and Sanford Peters and Van Slyke Am J Clin Path 3 412 1933 Quantitative Clinical Chemistry Vol I—Interpretations The Williams and Wilkins Co Baltimore 1932

Draw finger tip or oxalated blood to the first mark (0 025 cc ) on the hemoglobin pipet

Dilute with 0 1% sodium carbonate to the 5 025 cc graduation Assist in accurately stopping at the meniscus by slight pressure of the finger on the rubber tubing at tip of pipet

Expel directly into an absorption cell

Let stand 5 minutes

Read in Leitz Photrometer with filter 530

Determine concentration in grams from table

Calculate per cent hemoglobin as follows

$$\frac{\text{grams hemoglobin}}{\text{considered normal}} \times 100 = \textit{per cent}$$

Example

$$\frac{13\ 95}{15\ 5} \times 100 = 90\%$$

where 13 95 is the number of grams hemoglobin found and 15 5 is the considered equivalent of 100%




Fig 26—Leitz photrometer

## 7 Hellige CliniCol Procedure (*Courtesy Hellige Inc*)

*Hemoglobin (Oxyhemoglobin)*

Sheard and Sanford Am J Clin Path *3* 412 1933

1. Pipette exactly 4 0 ml Sodium Carbonate *0 1% (No R-319) into a clean dry absorption cell* No 904
2. Add 20 cmm fresh or oxalated blood Rinse pipette by drawing up and expelling some of the mixture several times If finger tip blood is used the specimen must be obtained without pressure from a free-flowing incision
3. Stir blood suspension thoroughly with a glass rod
4. Place approximately 5 ml distilled water in a second absorption cell for use as the blank
5. Rotate filter selector of CliniCol to filter No 520
6. Insert absorption cell containing the blank and set meter to 100 Important Absorption cells must always be inserted with a *frosted* side facing the operator
7. Replace absorption cell containing the blank with absorption cell containing the unknown
8. Note meter reading and obtain result in grams hemoglobin per 100 ml blood directly from hemoglobin (oxyhemoglobin) calibration chart

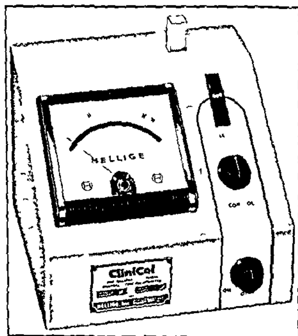



Fig 27 — Hellige CliniCol

8 BAUSCH & LOMB SPECTRONIC 20 PROCEDURE (*Courtesy Bausch & Lomb Co*)

*Hemoglobin*

*Cyanmethemoglobin Method*

1 Into 2 colorimeter tubes pipette exactly 5 ml Drabkin s solution (for preparation see Appendix)

2 In one tube add 0 02 ml whole blood (unknown) Rinse pipette several times by drawing some of the liquid into the pipette and expelling

3 Mix contents of tube by swirling

4 The other tube is the blank

5 Allow tubes to stand for 10 minutes

6 Set wavelength at 540 mmu and then place blank tube in instrument and set 100% T

7 Replace blank with unknown

8 Refer transmission reading to table to obtain concentration of sample in grams hemoglobin per 100 ml of blood

*Reference*

U S Armed Forces Medical Journal Vol V No 5 693 (May 1954)

*Notes*

1 Drabkin s solution contains cyanide When filling pipettes a suction bulb should be used

2 Drabkin s solution should not be used after precipitate has formed on bottom of storage bottle

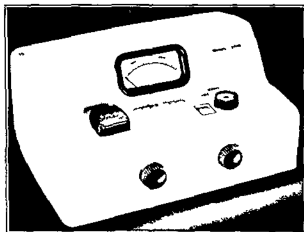


Fig 28 —Bau h & Lomb Spectronic 20

8 Bausch & Lomb Spectronic 20 Procedure (*Courtesy Bausch & Lomb Co*)

*Hemoglobin*

*Cyanmethemoglobin Method*

1. Into 2 colorimeter tubes pipette exactly 5 ml Drabkin's solution (for preparation see Appendix)
2. In one tube add 0.02 ml whole blood (unknown). Rinse pipette several times by drawing some of the liquid into the pipette and expelling
3. Mix contents of tube by swirling
4. The other tube is the blank
5. Allow tubes to stand for 10 minutes
6. Set wavelength at 540 mmu and then place blank tube in instrument and set 100% T
7. Replace blank with unknown
8. Refer transmission reading to table to obtain concentration of sample in grams hemoglobin per 100 ml of blood

*Reference*

U S Armed Forces Medical Journal Vol V No 5 (93 (May 1954)

10 COLEMAN SPECTROPHOTOMETER PROCEDURE (*Courtesy Coleman Instruments*)

*Hemoglobin (Cyanmethemoglobin)*

*Reference*


Stadie W C J Biol Chem *41* 237 1920

Drabkin D L and Austin J H J Biol Chem *98* 719 1932 *112* 51 (1935)

Crosby Munn and Furth U S Armed Forces Med J *5* 693 1954


*Procedure*

Measure exactly 5 0 ml of Drabkin s Reagent (for preparation see Appendix) into a test tube

Mix the blood sample thoroughly and transfer exactly 0 02 ml (Sahli pipette) into the reagent

Rinse the pipette three times with the reagent in the test tube

Allow the tube to stand for 10 minutes

Transfer a portion of the contents of the tube to a 12 × 75 mm cuvette and read in the Spectrophotometer at 540 mμ using a cuvette of Drabkin s Reagent as a reference blank

Read the hemoglobin value in gram per cent from the table

9 PHOTOVOLT LUMETRON PROCEDURE (*Courtesy Photovolt Corp*)

*Hemoglobin (Acid Hematin)*

Measure exactly 0 02 ml blood in a pipette (to contain) Wipe off outside of pipette

Blow the blood into exactly 10 ml of 0 1 N HCl in a colorimeter tube

Rinse the pipette by sucking up the solution and blowing it out

Stopper and mix

Read per cent transmission in LUMETRON after 3 minutes using green filter 530 against water as the blank set at 100% transmission

With the transmission value as obtained find result directly from Calibration Card which indicates per cent of normal opposite the transmission readings

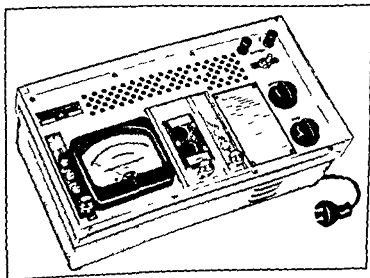


Fig 29 – Photovolt Lumetron

## DIFFERENTIAL WHITE CELL COUNT

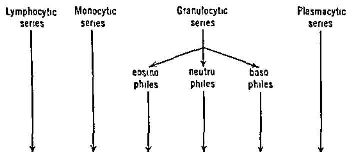
**Information Significant to the Student**

The differential white cell count is a determination of the different white cells in a patient's blood. The report often plays a large part in the diagnosis of disease. A brief discussion follows.

We classify men as to race. Thus we have the white race, yellow race, and black race. In a similar manner we classify the white cells as to series. Thus we have the lymphocytic series, monocytic series, granulocytic series, and plasmacytic series.

The white race may be divided into Americans, Europeans and Asiatics. In a similar vein the granulocytic series may be divided into eosinophiles, neutrophiles, and basophiles.

We may therefore picture 4 series of white cells, with one series being divided into 3 branches. Thus:




The people of each race start out as infants and go through various stages of development which we designate as child, juvenile, and adult. In a similar manner the cells of each series start out as infants and go through various stages of development. And just as we frequently take pictures of people at various stages of life, we can make drawings of cells at various stages of development. This has been done in Plate II where the young cells start out at the top of the

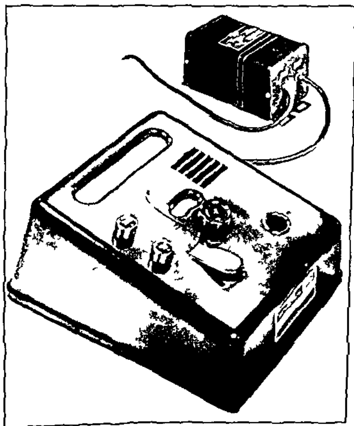
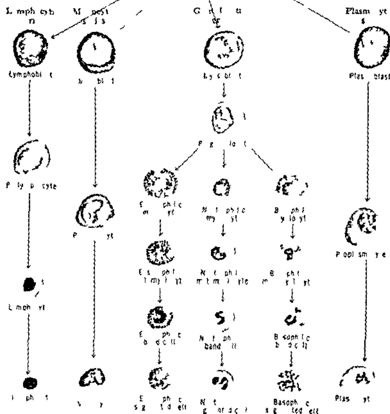


FIG. 30.—Coleman Spectrophotometer

PLATE II

CELL FORMING TISSUES

(Bone marrow liver and spleen)



page and increase in maturity as they move to the bottom of the page

The cells in the vast upper portion of the chart are the very young cells. These are usually confined to their birth place in the bone marrow, liver and spleen. After 5-days development they are eager to battle the diseases of the body. Therefore they pass into the blood stream and serve as sentinels of sanitation cruising through the body in constant search of combat.

In a single drop of normal blood there are thousands of white cells. In this same drop however there are only six different white cells. And the student who is about to study these cells should pause to become acquainted with their names and characteristics. Their names are given below and their characteristics are illustrated in Plate II.

Monocytes
Lymphocytes
Eosinophilic segmented cells
Basophilic segmented cells
Neutrophilic segmented cells
Neutrophilic band cells

The above cells are not present in equal numbers. For example there are more lymphocytes than monocytes. When we count 100 white cells and report the number of each type present we perform a differential white cell count.

Since we count 100 white cells the values for the differential white cell count are given in per cent. For example if we find 30 monocytes and 70 lymphocytes in 100 white cells we report 30 per cent monocytes and 70 per cent lymphocytes. The normal values for the differential white cell count are given in Table 6.

In disease the percentages may vary. For example the normal values for lymphocytes are 20 to 35 per cent. But in lymphocytic leukemia we may find 95 per cent lymphocytes, 5 per cent neutrophilic segmented cells and none of the other cells. The increased cell percentage which is found in the more common diseases is given in Table 7.

It is apparent that the differential white cell count is a useful diagnostic aid and the physician may use it to confirm or refute a questionable diagnosis. To illustrate, the physician may have the following problem. Does the patient have a simple stomachache or appendicitis? If the differential

***Table 6.—Normal Values for a Differential White Cell Count***

| *Cell* | *Per Cent Found* |
|---|---|
| Basophilic segmented cells | 0 to 1 |
| Eosinophilic segmented cells | 1 to 3 |
| Neutrophilic band cells | 2 to 6 |
| Monocytes | 2 to 6 |
| Lymphocytes | 20 to 35 |
| Neutrophilic segmented cells | 55 to 75 |

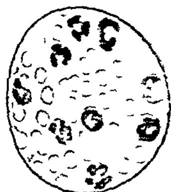
***Table 7.—Increased Cell Percentages Found in the More Common Diseases***

| *Increased Per Cent of Lymphocytes* | *Increased Per Cent of Monocytes* | *Increased Per Cent of Eosinophilic Cells* | *Increased Per Cent of Neutrophilic Cells* |
|---|---|---|---|
| Mumps | Typhus | Asthma | Appendicitis |
| Whooping cough | Brucellosis | Hay fever | Pneumonia |
| Infectious mononucleosis | Tuberculosis | Scarlet fever | Tonsillitis |
| Lymphocytic leukemia | Monocytic leukemia | Parasitic infestations | Meningitis |
| | | | Diphtheria |
| | | | Granulocytic leukemia |

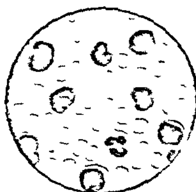
white cell count is normal, the physician rules out appendicitis. On the other hand, if the differential white cell count shows about 85 per cent neutrophilic segmented cells and 15 per cent neutrophilic band cells, the physician may operate.

The suffix *-osis* on the end of a word means *an increase in*. Thus, when the percentage of lymphocytes is increased the

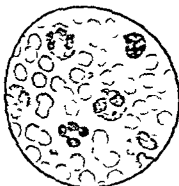
PLATE III



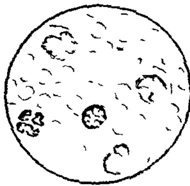
[illegible] Leukocytosis



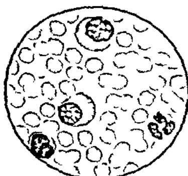
L[illegible]



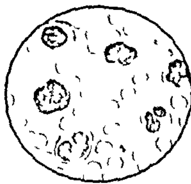
Eosinophilic Leukocytosis



M[illegible]



H[illegible] C[illegible] R[illegible]



Infecti[illegible] M[illegible]nucl[illegible]sis

VARIOUS TYPES OF LEUKOCYTOSIS
(From M[illegible] S[illegible] A Text[illegible] Clinical [illegible] 5th edition [illegible]
Williams & Wilkins Co.)

*condition is referred to as a lymphocytosis* By a similar token we have a *monocytosis, eosinophilic leukocytosis* and *neutrophilic leukocytosis* *

The cells which the technician may expect to find in the various types of leukocytosis are illustrated in Plate III The numerous small pink objects surrounding the white cells are red cells The cells in all the illustrations have been stained and magnified 1000 times

There are two parts to each white cell The central portion known as the nucleus and the portion surrounding the nucleus which is known as the *cytoplasm* And just as people are largely identified by the characteristics of their face and body the white cells are largely identified by the characteristics of their nucleus and cytoplasm Before turning to the procedure for the differential white cell count the student should carefully study the cells in Plate III and note the following

The illustration labelled *Lymphocytosis* contains 1 neutrophilic segmented cell and 8 lymphocytes Note that there are both small and large lymphocytes

The illustration labelled *Monocytosis* contains 1 neutrophilic segmented cell 1 lymphocyte and 3 monocytes Note that the monocytes are the largest cells and also that they have a sprawling nucleus

The illustration labelled *Neutrophilic Leukocytosis* contains 3 neutrophilic band cells and 4 neutrophilic segmented cells Note that the nucleus of the band cell is shaped like a band On the other hand the nucleus of the segmented cell is broken up into segments These segments are separated by a fine filament The unbroken or non filamented band in the neutrophilic band cell is the only characteristic which distinguishes it from the neutrophilic segmented cell

The illustration labelled *Eosinophilic Leukocytosis* contains 1 neutrophilic segmented cell 1 lymphocyte 3 eosinophilic segmented cells and 1 ruptured eosinophilic segmented cell The identifying characteristic of the eosinophiles is the coarse red granulation in the cytoplasm

* An interpretation of the words which are used in hematology is given in the Appendix on page 311

The illustration labelled *Infectious Mononucleosis* shows the abnormal lymphocytes seen in this particular disease Note the moth-eaten appearance of the cytoplasm

The illustration labelled *Plasma Cell Reaction* shows plasma cells These are seldom seen They may be found in the blood however in measles chicken pox multiple myeloma plasmacytic leukemia and serum sickness

**Procedure for the Differential White Cell Count**

It is strongly urged that the student first read this entire procedure through and then make a differential white cell count by following the summary on page 90

The blood for the differential white cell count is obtained from a finger puncture or venipuncture The steps in the procedure are outlined below and a detailed explanation of each step follows

1 Making the blood smear
2 Staining the cells
3 Identifying the cells
4 Classifying the cells

1 *Making the Blood Smear*

There are two methods of making a blood smear slide method and coverglass method The slide method is illustrated in Figure 31 and the coverglass method is illustrated in Figure 32

The slide method is the simplest and consequently has become the most popular In this method the criteria for a well made smear is as follows

*a* The blood must *not* cover the entire portion of the slide
*b* There should be a thick portion and a thin portion
*c* The thick portion should make a gradual transition to the thin portion (Fig 33)

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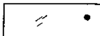
1 Making the blood smear
2 Staining the cells
3 Identifying the cells
4 Classifying the cells

1 *Making the blood Smear*

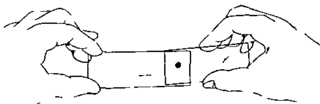
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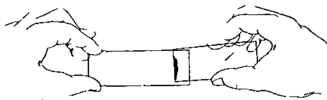
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*b* There should be a thick portion and a thin portion
*c* The thick portion should make a gradual transition to the thin portion (Fig 33)



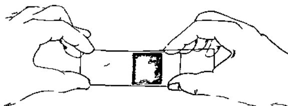
1 Place the slide on a table or flat surface Place a *small* drop of blood on the slide



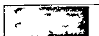
2 Using another slide as a spreader place one edge of the spreader on the slide at about a 30 degree angle (this means that the other edge of the spreader will be about an inch above the table) Now draw spreader toward the drop



3 Contact the drop of blood



4 With a rapid smooth gliding motion push the spreader over the slide in the direction of the arrows



5 Completed blood slide

FIG 31 —Slide method of making a blood smear

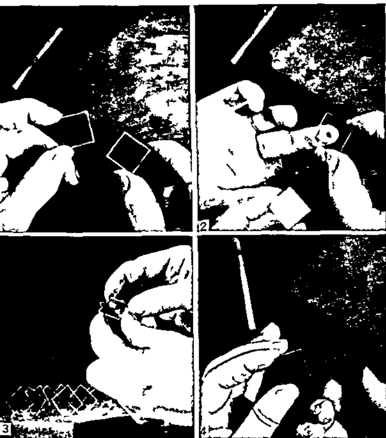
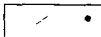
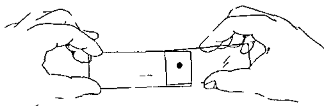


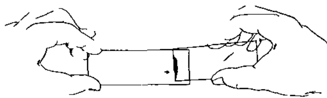
Fig. 32.—Cover glass method of making a blood smear. 1, A cover glass ([illegible] inch No. 2) is held at the adjacent corners with the thumb and forefinger of each hand. 2, the drop of blood is touched with the cover glass held in the right hand. 3, the cover glass carrying the drop of blood is quickly placed parallel on the cover glass held in the left hand. 4, after the blood has spread by capillary attraction, the cover glasses are drawn apart with a steady motion, care being taken to keep them parallel. After drying in the air, the films are ready for staining. The drop of blood must be globoid and just large enough to cover the cover glass when properly spread. (Haden, *Clinical Laboratory Methods*, courtesy of C. V. Mosby Company.)



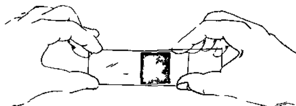
1 Place the slide on a table or flat surface Place a *small* drop of blood on the slide



2 Using another slide as a spreader place one edge of the spreader on the slide at about a 30 degree angle (this means that the other edge of the spreader will be about an inch above the table) Now draw spreader toward the drop



3 Contact the drop of blood



4 With a rapid smooth gliding motion push the spreader over the slide in the direction of the arrows



5 Completed blood slide

FIG. 31 —Slide method of making a blood smear

The white cells are largely identified by their preference for the above dyes. And in some cases the cells are even named for the dye which they prefer. For example cells which prefer the acid dye (eosin) are called eosinophiles—meaning love of eosin. Whereas cells which prefer the basic dye are called basophiles. And cells which prefer a mixture of the acid and basic dye are called neutrophiles.

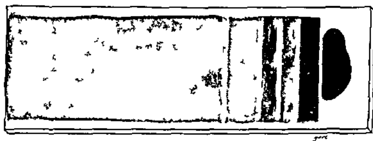
In the staining process a buffer solution is used to control the acid base balance of the stain. This is a most important function. If the buffer solution is too acid it makes the acid dye too bright and the basic dye too faint. On the other hand if the buffer solution is too basic it makes the basic dye too bright and the acid dye too faint. In either case the result is a poorly stained slide.

The acid base balance of a solution is measured by the pH value. And a buffer solution should have a pH value between 6.4 and 6.8. This allows the best color contrast between the acid dye and basic dye. The directions for preparing the buffer solution and Wright's stain are given in the Appendix.

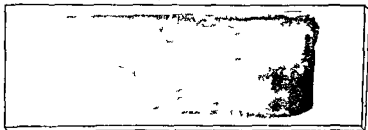
The procedure for staining a blood slide is given below.

1. Place the slide on the rack of a staining dish.
2. Add 25 to 40 drops of Wright's stain. This should cover the entire slide.
3. Let stand 1 minute.
4. Add about 20 drops of buffer solution so that it is evenly distributed over the slide (Fig. 34).
5. Mix the two solutions by blowing gently on several portions of the slide (Fig. 34). When the solutions are well mixed a silvery scum usually floats on top of the solution.
6. Allow to set for 4 to 8 minutes. The time varies because each batch of Wright's stain has a different staining time.
7. Remove the excess staining solution from the slide by flooding freely with *distilled* water.

After the blood smear is made it should be allowed to dry. This takes a few minutes. The patient's name should then be written on the smear with an ordinary pen or pencil (Fig 31 and Fig 33). This of course is done for the purpose of identification. And it should never be neglected for blood smears could easily get mixed up and cause an incorrect diagnosis.



POOR SMEAR




GOOD SMEAR

FIG 33 —Good and poor blood smears

*2 Staining the Cells*

The cells are stained with one of the following stains: Wright's, Giemsa or Wilson. Of these stains the most popular is Wright's.

Wright's stain is a methyl alcohol solution of an acid dye and a basic dye. The acid dye is known as eosin; it is red in color. The basic dye is known as methylene blue; it is blue in color.

## *Table 8.—Recommended Nomenclature for the White Cells*

The essential characteristics which distinguish each stage of development from the preceding and following ones are given in italics above and below the arbitrary lines of demarcation. (Courtesy of the American Medical Association)

| *Lymphocytic Series* | *Monocytic Series* | *Granulocytic Series* | *Plasmacytic Series* |
|---|---|---|---|
| Lymphoblast | Monoblast | Myeloblast | Plasmablast |
| *Fine chromatin structure* | *Fine chromatin structure* | *Fine chromatin structure* | *Fine chromatin structure* |
| *Chromatin clump* | *Irregularly shaped nucleus* | *Chromatin clump* | *Chromatin clump* |
| Prolymphocyte | Promonocyte | Progranulocyte | Proplasmacyte |
| *Large cell size or finer chromatin* | *Nucleolus present* | *No specific granules* | *Nucleolus present* |
| *Coarse chromatin structure* | *Nucleoli absent* | *Specific granules* | *Nucleoli absent* |
| Lymphocyte | Monocyte | Myelocyte | Plasmacyte |
| | | *Round or oval nucleus* | |
| | | *Indented nucleus* | |
| | | Metamyelocyte | |
| | | *Bean or kidney shaped nucleus* | |
| | | *Part of nucleus with parallel sides* | |
| | | Band cell | |
| | | *No filament present* | |
| | | *Nuclear lobes connected by filaments* | |
| | | Segmented cell | |

8 Remove from rack wipe bottom of slide stand on end and allow to dry
9 NOTE If the stain has been left on too long and the cells are over stained the stain may be removed with 95 per cent alcohol and the slide restained

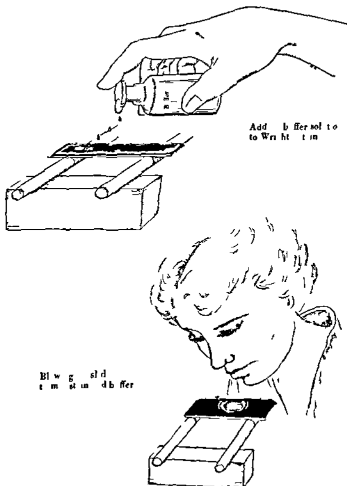


FIG 34 —Staining the blood slide

## *Table 9—System of Questions for Identification of White Cells*

(Modified from Naval Medical School Manual)

I What is the size of the cell Is it
  *a* Small as a red cell (7 5 microns)
  *b* Medium as a segmented cell (15 microns)
  *c* Large as a monocyte? (20 microns)

II What is the size and shape of the nucleus Is it
  *a* Large folded irregular coarse clumps and strands with no apparent nuclear membrane as in the monocyte
  *b* Relatively small and round with nuclear membrane as in the lymphocyte?
  *c* Relatively large and pale with fine chromatin network containing nucleoli as in the blasts and pro cells?
  *d* Round and covered with granules as in the myelocyte?
  *e* Slightly indented as in the metamyelocyte
  *f* Horse-shoe shaped as in the band cell
  *g* Lobed as in the segmented cell?

III What is the color of the cytoplasm
  *a* Translucent pale sky blue as a lymphocyte
  *b* Opaque gray blue as a monocyte
  *c* Pink as a neutrophilic cell?
  *d* Washed out blue or colorless as an eosinophilic cell
  *e* Light blue as a basophilic cell?
  *f* Deep blue as a blast cell or plasma cell

IV Are there any granules in the cytoplasm? If so are they
  *a* Fine and diffuse as in the monocyte
  *b* Coarse and clumped as in the large lymphocyte and myelocyte?
  *c* Lilac-colored as in the neutrophilic cell?
  *d* Orange-red and coarse as in the eosinophilic cell?
  *e* Purplish black coarse and irregular as in the basophilic cell

V What is the texture of the nucleus or cytoplasm? Is it the
  *a* Smooth chromatin network or finely stippled appearance of the nucleus of the blast cells
  *b* Loose smooth network of the nucleus of stem cells?
  *c* Dense hill and valley effect of the nucleus of mature lymphocytes granulocytes plasmacytes and nucleated red cells
  *d* Filmy translucent appearance of the nucleus of monocytes?
  *e* Dense texture of the cytoplasm of blast cells?
  *f* Denser metallic appearance of the cytoplasm of nucleated red cells
  *g* Monocytic cytoplasm resembling light blue cotton bed sheet?

3 *Identifying the White Cells*

It is very difficult to learn the identification of cells from a textbook The student should have the help of an instructor This may be supplemented by reference to Plates II and III and the recommended nomenclature given in Table 8

As an aid in identifying each cell, the following three questions should always be kept in mind

*a* What is the size of the cell?
*b* What are the characteristics of the nucleus?
*c* What are the characteristics of the cytoplasm?

After the student has seen the various cells under the microscope the system of questions in Table 9 may help identify cells which seem difficult to recognize For part V of this table we are indebted to Dr R U Northrip

The directions for making the differential white cell count are given below

1 Place a drop of oil on the *thin* portion of the stained blood slide
2 Place the slide on the stage of the microscope Now lower the oil immersion (95 ×) objective into this drop of oil
3 First check to see that the condenser is moved up as close as possible to the slide *This insures the maximum amount of light* Now adjust the mirror so that the light is centered up through the eye piece
4 Using the coarse and fine adjustments bring the cells into focus
5 Observe the sample differential white cell count shown in Figure 35
6 By moving the slide in the path of the broken arrow shown in Figure 36 identify and record the first 100 white cells you see Do not skip any If there are some you are not sure of ask for help or record them as unidentified

two the filament and non filament classification An explanation of each method follows

SCHILLING CLASSIFICATION

Schilling was one of the first to notice that in many diseases there is an increase in the percentage of immature neutrophiles of the granulocytic series His blood chart reported the percentages of the various cell types and—in part—was arranged in the following manner

| | Neutrophilic Myelocytes | Neutrophilic Metamyelocytes | Neutrophilic Band Cells | Neutrophilic Segmented Cells |
|---|---|---|---|---|
| Normal values | 0% | 0% | 2 to 6% | 55 to 75% |

Note that the immature cells are on the left side of the chart If the percentage of these immature cells became increased Schilling called it a shift to the left

When the shift to the left was accompanied by a low white cell count he called it a *degenerative* shift to the left This is found in such diseases as typhoid fever It is due to a depression of the cell factories in the bone marrow

When the shift to the left was accompanied by a high white cell count Schilling called it a *regenerative* shift to the left This is found in infectious diseases It is caused by a stimulus of the cell factories in the bone marrow

The Schilling classification of the differential white cell count has become quite popular The cell types which are reported and the normal percentages are given in Table 10

*Table 10 —Schilling Classification of the Differential White Cell Count*

| Cell | Per Cent |
|---|---|
| Neutrophilic myelocyte | 0 |
| Neutrophilic metamyelocytes | 0 |
| Neutrophilic band cells | 2 to 6 |
| Neutrophilic segmented cell | 55 to 75 |
| Lymphocytes | 20 to 35 |
| Monocyte | 2 to 6 |
| Eosinophilic segmented cells | 1 to 3 |
| Basophilic segmented cells | 0 to 1 |

| Cell | Count | Total Number |
|---|---|---|
| Neutrophilic band cells | IIII | 4 |
| Neutrophilic segmented cells | 𝍸 𝍸 𝍸 𝍸<br>𝍸 𝍸 𝍸 𝍸<br>𝍸 𝍸 𝍸 𝍸 | 60 |
| Lymphocytes | 𝍸 𝍸 𝍸 𝍸<br>𝍸 IIII | 29 |
| Monocyte | 𝍸 | 5 |
| Eosinophilic segmented cell | II | 2 |
| Basophilic segmented cells | 0 | 0 |
| | | 100 |

Fig 35 —Simple differential white cell count

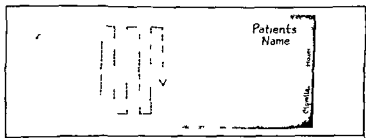



Fig 36 —Method of examining the blood slide for the differential white cell count When a blood slide is made the large cells tend to accumulate on the edge of the slide whereas the small cells tend to stay in the middle of the slide It is apparent that if the cells are counted only on the edge or in the middle of the slide it would not be a true representative sample of the patient s cells The slide is therefore examined by following the path of the broken arrow shown above This is accomplished by moving the slide with the movable stage

## 4 *Classifying the White Cells*

When 100 white cells have been identified and recorded the report of the differential white cell count is given according to either of the following one the Schilling classification

1 Make a blood smear
   *a* Place a small drop of blood on a glass slide
   *b* Using another slide as a spreader spread the drop over the slide
   *c* Allow to dry
   *d* Write the patient s name on the slide
2 Stain the cells
   *a* Place slide on rack
   *b* Add 25 to 40 drops of Wright s stain
   *c* Let stand 1 minute
   *d* Add about 20 drops of buffer solution
   *e* Mix by blowing on slide
   *f* Let stand 4 to 8 minutes
   *g* Wash and allow to dry
3 Identify the white cells
   *a* Place slide on stage of microscope
   *b* Using the oil immersion objective iden tify and record 100 white cells
4 Classify cells according to Schilling classifi cation
   *a* Report the number or percentage of each cell present

## EXAMINATION OF THE STAINED RED CELLS

**Information Significant to the Student**

Examination of the stained red cells consists of inspecting the red cells of the blood slide for variations in size shape hemoglobin content and abnormal inclusions In many diseases particularly the anemias these variations are quite marked and become significant to the physician during diagnosis and treatment

In pernicious anemia for example the red cells are very large In the anemia of pregnancy the red cells are pale And in sickle cell anemia they are shaped like a sickle or crescent

The descriptive terms for abnormal red cells are given in Table 12

FILAMENT AND NON FILAMENT CLASSIFICATION

This classification reports the percentage of each cell with the exception of the neutrophiles of the granulocytic series This of course is the only manner in which it differs from the Schilling classification The neutrophiles of the granulocytic series are reported as follows

The mature neutrophiles of the granulocytic series are the neutrophilic segmented cells These make up the filament count

The immature neutrophiles of the granulocytic series are the neutrophilic myelocytes metamyelocytes and band cells These are grouped together and their total percentage make up the non filament count For example in appendicitis we might find 0 neutrophilic myelocytes 2 neutrophilic metamyelocytes and 8 neutrophilic band cells This would make a non filament count of 10 per cent

The cells reported and the normal values for the filament and non filament classification are given in Table 11

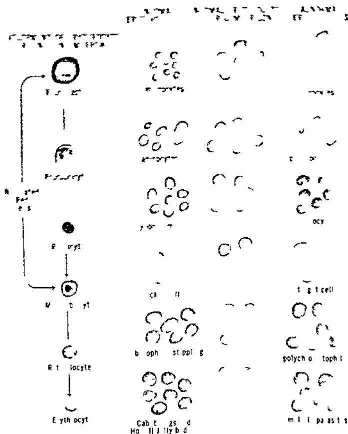
*Table 11 —Filament and Non Filament Classification of the Differential White Cell Count*

| *Cell* | | *Per Cent* |
|---|---|---|
| Neutrophilic myelocytes<br>Neutrophilic metamyelocytes<br>Neutrophilic band cells | Non filamented cells | 2 to 6 |
| Neutrophilic segmented cells | | 55 to 75 |
| Lymphocytes | | 20 to 35 |
| Monocytes | | 2 to 6 |
| Eosinophilic segmented cells | | 1 to 3 |
| Basophilic segmented cells | | 0 to 1 |

*Summary of the Differential White Cell Count*

After reading the foregoing discussion perform a differential white cell count by following the steps in the summary below If you have trouble with any of the steps simply refer back to the detailed discussion

# PLATE IV



NORMAL AND ABNORMAL RED CELL

*Table 12—Descriptive Terms for the Red Cells*

| *Descriptive Word* | *Meaning* | *Where Found* |
|---|---|---|
| Microcyte | Small cell | Microcytic anemias |
| Normocyte | Normal cell | Normal blood |
| Macrocyte | Large cell | Pernicious anemia |
| Anisocytosis | Variation in size of cells | Many types of anemia |
| Poikilocytosis | Variation in shape of cells | Many types of anemia |
| Hypochromia | Deficient in hemoglobin | Hypochromic anemias |
| Normochromic | Normal in hemoglobin | Normal blood |
| Polychromatophilia | Variation in color of cells | Many types of anemia |
| Sickle cell | Shaped like a sickle or crescent | Sickle cell anemia |
| Spherocyte | Small round cell well packed with hemoglobin | Congenital hemolytic anemia |
| Target cell | Deficient in hemoglobin with a bull s eye in center of cell | Some cases of obstructive jaundice |
| Howell Jolly bodies | Nuclear remnants shaped like dots | Severe anemias |
| Cabot rings | Nuclear remnants shaped like a ring | Severe anemias |
| Basophilic stippling | Coarse dark violet granulation | Lead poisoning |

These abnormal red cells and the stages in the development of a red cell are illustrated in Plate IV

**Procedure for the Examination of the Stained Red Cells**

Choose a thin portion of the same blood slide which was used for the differential white cell count and examine the red cells for any variation in size shape hemoglobin content and other abnormalities such as malaria parasites

Report all abnormalities and qualify your descriptive terms with the adjectives slight moderate or marked A sample report found in an anemia is given below

> Red cells show a marked degree of hypochromia a moderate degree of anisocytosis and a slight degree of poikilocytosis

## Chapter 3

# Further Examinations of the Red Cells

IN THE complete blood count the red cells are counted and the blood slide is inspected for any red cell abnormalities. This information, however, is not sufficient for the diagnosis and treatment of many types of anemia, and further examinations of the red cells are required. For example, the red cells are more fragile in hemolytic jaundice than in obstructive jaundice, and the red cell fragility test becomes a considerable aid in the differential diagnosis of these two diseases. The demonstration of sickle shaped red cells is usually sufficient evidence for a diagnosis of sickle cell anemia. In several other anemias the physician is interested in the body's ability to produce red cells. A reticulocyte count furnishes this information.

This chapter presents those supplementary examinations which aid the physician in the diagnosis and treatment of *the anemias. Although the sedimentation rate is not useful* in this connection, it is included in this chapter since it is an examination of the red cells. The tests will be considered in the following order:

- Sedimentation rate
- Hematocrit reading
- Sickle cell examination
- Fragility test
- Reticulocyte count
- Red cell indices

## SEDIMENTATION RATE OF RED CELLS

### Information Significant to the Student

When the red cells are allowed to settle out from their plasma, the speed of their fall is known as the sedimentation

3 Shake the test tube gently to obtain an even distribution of cells in the plasma fill the Westergren tube to the zero mark and place in the Westergren rack (Fig 37) Failure to mix the blood before filling the Westergren tube may cause a considerable error because

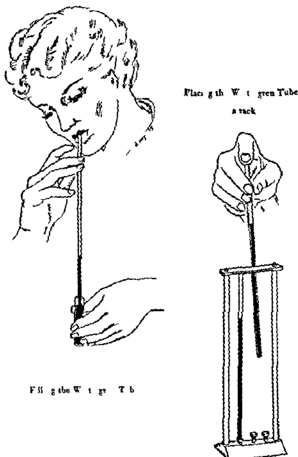



Fig 37 —*Filling the Westergren tube and placing it in a rack*

rate Although it is not specific for any particular disease the sedimentation rate is considered a useful diagnostic aid It is increased in acute infections rheumatic fever tuberculosis pregnancy nephritis and cancer

The normal values for the various methods of determination given in millimeters per hour are listed in the table below Note that the Cutler method has lower normal values than the other two methods Therefore when reporting the sedimentation rate the technician should always specify the method of determination and the normal values

*Table 13 —Normal Values for the Sedimentation Rate*

| | *Westergren* | *Cutler* | *Wintrobe Landsberg* |
|---|---|---|---|
| Men | 0–9 mm | 0–8 mm | 0–9 mm |
| Women | 0–20 mm | 0–10 mm | 0–20 mm |

## Procedures for the Sedimentation Rate

The sedimentation rate is determined by filling a graduated tube with oxalated or citrated blood and recording the rate at which the red cells settle out from their plasma The Westergren Cutler and Wintrobe Landsberg methods are widely used The procedures follow

In performing this test the sedimentation rate tube should always be placed in an *exact* vertical position Deviations from this position will greatly increase the sedimentation rate

### Westergren Method

1 Get materials for a venipuncture In a test tube place 0 5 cc of 3 8 per cent sodium citrate (preparation given in the Appendix on page 242)
2 Make a venipuncture add 4 5 cc of blood to the test tube and mix by inverting the tube several times (In order to measure exactly 4 5 cc some technicians withdraw about 5 cc of blood remove the needle hold several gauze pads over the neck of the syringe and force blood out until it reaches the 4 5 mark )

3 Shake the test tube gently to obtain an even distribution of cells in the plasma fill the Westergren tube to the zero mark and place in the Westergren rack (Fig 37) Failure to mix the blood before filling the Westergren tube may cause a considerable error because

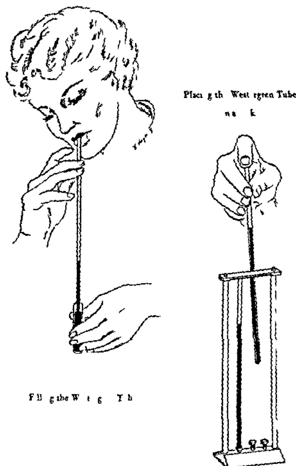



Fig 37 —Filling the Westergren tube and placing it in a rack

the cells have already begun to settle out from their plasma

4 In exactly 1 hour, record the number of millimeters that the cells have fallen

*Normal Values*

Men — 0-9 mm in 1 hour
Women — 0-20 mm in 1 hour

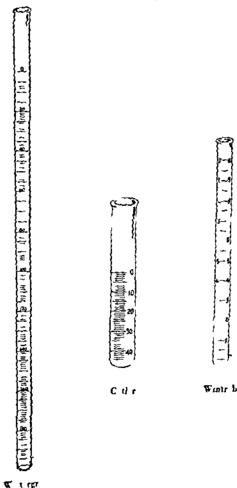



Fig 38 — Sedimentation rate tube

**Cutler Method**

1 Draw 0.5 cc of a sterile 3.8 per cent sodium citrate solution (preparation given in Appendix on page 242) into a sterile 5 cc syringe
2 With the same syringe make a venipuncture and withdraw 4.5 cc of blood Mix the anticoagulant and blood by inverting the syringe several times
3 Fill a Cutler tube (Fig 38) to the zero mark
4 Make readings every 10 min for a period of 1 hour
5 Plot the readings on a Cutler sedimentation rate graph (Fig 39)

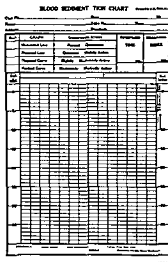


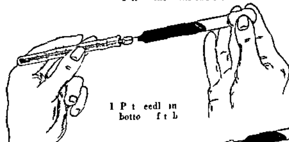
Fig 39 —Cutler sedimentation rate graph

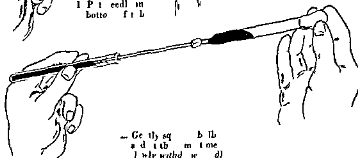
*Normal Values*

Men — 0–8 mm in 1 hour
Women — 0–10 mm in 1 hour

**Wintrobe Landsberg Method**

1 Get materials for a venipuncture and an oxalated test tube (preparation given in Appendix on page 241)





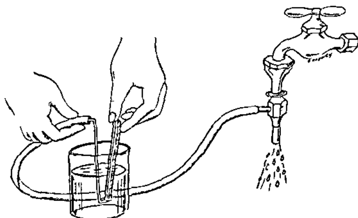


Fig 40 – Filling and cleaning a Wintrobe tube

2 Make a venipuncture withdraw 5 cc of blood and place in the oxalated test tube Cover the mouth of the test tube and invert several times to dissolve the oxalate
3 Shake the test tube gently to obtain an even distribution of cells in the plasma Failure to mix the blood before filling the Wintrobe tube may cause a considerable error because the red cells have already begun to settle out from their plasma
4 Using either a capillary tube or needle and syringe fill the Wintrobe tube to the zero mark (Fig 40)
5 Place the tube in a Wintrobe rack so that the tube is *exactly* vertical
6 In exactly 1 hour read the number of millimeters that the cells have fallen The sedimentation rate scale starts with a 0 and read downward It has a total range of 10 cm or 100 mm The other scale on the tube is for the hematocrit reading

*Normal Values*

Men — 0–9 mm in 1 hour
Women — 0–20 mm in 1 hour

In anemias it may be necessary to make a correction for the decreased number of red cells The procedure for the correction follows

**Procedure for Correcting the Sedimentation Rate**

After the reading of the sedimentation rate has been made centrifuge the tube until the cells are so completely packed that further centrifuging does not lower their volume If centrifuged at 3 000 revolutions per minute this usually takes about 30 minutes The scale on the right side of the Wintrobe tube is 10 cm or 100 mm in length Using this scale and reading *upward* from the bottom of the tube take

the reading in millimeters of packed red cells With the aid of the chart in Figure 41 make the correction as follows

Place your pencil on the horizontal line corresponding to your reading of the sedimentation rate Move to the right until you strike the vertical line corresponding to your read

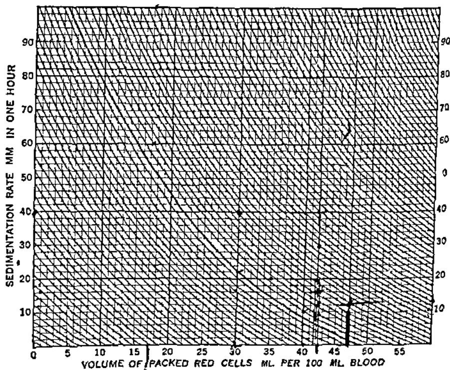



Fig 41 —Wintrobe-Landsberg chart for correction of the sedimentation rate (Wintrobe and Landsberg Am J Med S i)

ing of the packed red cells Now place your pencil on the curved line nearest this point If the patient is a woman follow this curved line downward until you strike the vertical line 42 The value of the horizontal line at this point is the corrected sedimentation rate If the patient is a man follow

the curved line downward until you strike the vertical line 47 The value of the horizontal line at this point is the corrected sedimentation rate

To illustrate if you have the following data

| | |
|---|---|
| Patient | woman |
| Reading of the sedimentation rate | 40 mm |
| Reading of the packed red cells | 30 mm |

The corrected sedimentation rate is found as follows Place your pencil on the horizontal line 40 (reading of sedimentation rate) Move to the right until you strike the vertical line 30 (reading of packed red cells) Follow the nearest curved line downward until you strike the vertical line 42 (end point for women) The value of the horizontal line at this point is 18 This is the corrected sedimentation rate If the patient *were a man you would have followed the curved line* downward until you struck the vertical line 47 (end point for men) The value of the horizontal line at this point is 12 This would be the corrected sedimentaiton rate for men

Make the report as follows

| | |
|---|---|
| Uncorrected sedimentation rate | 40 mm |
| Reading of packed red cells | 30 mm |
| Corrected sedimentation rate | 18 mm |
| Method of Wintrobe | |

## HEMATOCRIT READING

**Information Significant to the Student**

When blood is centrifuged the per cent occupied by the packed red cells is known as the hematocrit reading For example if 10 cc of blood are centrifuged and the packed red cells occupy 5 cc the hematocrit reading is 50 per cent

The average man has a hematocrit reading of 45 per cent and the average woman has a hematocrit reading of 42 per cent The hematocrit reading is low whenever the red cell

count or hemoglobin is low Consequently, the hematocrit reading is low in all types of anemia

A high hematocrit reading may be found in dehydration conditions In such cases fluid has been lost and the blood contains more cells than plasma Some of the above mentioned readings are illustrated in Figure 42 and the normal hematocrit values are given in Table 14

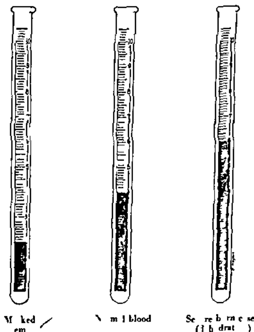


FIG 42 —Low normal and high hematocrit reading The lower darker portion of each tube is red cells the upper lighter portion is plasma

*Table 14 —Normal Values for the Hematocrit Reading*

| | Normal Value | Average Normal Values |
|---|---|---|
| Men | 40–50% | 45% |
| Women | 37–47% | 42% |

**Procedures for the Hematocrit Reading**

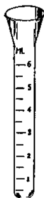
The hematocrit reading is made by centrifuging a sample of blood and calculating the per cent occupied by the packed red cells The white cells and platelets form a very fine white layer on top of the packed red cells but their volume is so small that it does not interfere in the reading The Wintrobe Sanford Magath and Van Allen methods are used to make the determination The procedures follow

**Wintrobe Method**

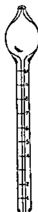
1 Get materials for a venipuncture and an oxalated test tube (preparation given in Appendix on page 241)
2 Make a venipuncture withdraw 5 cc of blood and transfer it to the oxalated tube Cover the mouth of the tube and invert several times to dissolve the oxalate



Wintrobe



Sanford Magath



Van Allen

Fig 43 —Hematocrit tubes

3. Shake the tube gently to get an even distribution of cells in the plasma (Failure to do this may cause a considerable error.) Using either a capillary tube or needle and syringe fill the Wintrobe tube (Fig 43) to the 10 cm mark
4. Centrifuge at 3 000 revolutions per minute for 30 minutes—or centrifuge at high speed until two successive spinnings give the same reading (This simply indicates that the cells have been well packed.)
5. Calculation Use the scale on the right side of the tube It is 10 cm in length and starts at the bottom of the tube and reads up Read the number of cm corresponding to the level of packed red cells and calculate the per cent For example if the reading is 4.2 cm the hematocrit reading is found as follows

$$\frac{4.2 \text{ cm (packed cells)}}{10.0 \text{ cm (blood)}} \times 100 = 42\%$$

*Normal Values*

| | |
|---|---|
| Men | 40–50% |
| Women | 37–47% |

**Sanford Magath Method**

1. Place exactly 1 cc of a 1.1% solution of sodium oxalate (preparation given in Appendix on page 242) in a Sanford Magath tube (Fig 43) Get materials for a venipuncture
2. Make a venipuncture withdraw about 8 cc of blood and fill the Sanford Magath tube exactly to the 6.0 cc mark Mix by inverting the tube several times
3. Centrifuge at 3 000 revolutions per minute for 30 minutes—or centrifuge at high speed until two successive spinnings give the same reading

4 Read the level of packed red cells and calculate the percentage For example if the reading is 2 2 cc the hematocrit reading is found as follows

$$\frac{2\,2\text{ cc (packed cells)}}{5\,0\text{ cc (blood)}} \times 100 = 44\%$$

*Normal Values*

| | |
|---|---|
| Men | 40–50% |
| Women | 37–47% |

**Van Allen Method**

1 Get a Van Allen tube (Fig 43) and a bottle of 1 3% sodium oxalate solution (preparation given in Appendix on page 242)
2 Make a rather deep finger puncture discard the first drop and draw blood exactly to the 10 mark of the Van Allen tube Immediately draw the sodium oxalate solution into the tube until the bulb is about half full of the mixture of blood and sodium oxalate Using either the clip that comes with the tube or a broad rubber band close the ends of the tube
3 Centrifuge at 3 000 revolutions per minute for 30 minutes—or centrifuge at high speed until two successive spinnings give the same reading
4 Calculations The scale is 100 units Starting at the bottom of the tube and reading up note the number of units occupied by the packed red cells Calculate the percentage For example if the packed red cells occupy 40 units the hematocrit reading is found as follows

$$\frac{40\text{ units (packed red cells)}}{100\text{ units (blood)}} \times 100 = 40\%$$

3 Shake the tube gently to get an even distribution of cells in the plasma (*Failure to do this may cause a considerable error*) Using either a capillary tube or needle and syringe fill the Wintrobe tube (Fig 43) to the 10 cm mark

4 Centrifuge at 3 000 revolutions per minute for 30 minutes—or centrifuge at high speed until two successive spinnings give the same reading (This simply indicates that the cells have been well packed)

5 Calculation Use the scale on the right side of the tube It is 10 cm in length and starts at the bottom of the tube and reads up Read the number of cm corresponding to the level of packed red cells and calculate the per cent For example if the reading is 4 2 cm the hematocrit reading is found as follows

$$\frac{4.2 \text{ cm (packed cells)}}{10.0 \text{ cm (blood)}} \times 100 = 42\%$$

*Normal Values*

| | |
|---|---|
| Men | 40–50% |
| Women | 37–47% |

**Sanford Magath Method**

1 Place exactly 1 cc of a 1 1% solution of sodium oxalate (preparation given in Appendix on page 242) in a Sanford Magath tube (Fig 45) Get material for a venipuncture

2 Make a venipuncture withdraw about 8 cc of blood and fill the Sanford Magath tube exactly to the 6 cc mark Mix by inverting the tube several times

3 Centrifuge at 3 000 revolutions per minute for 30 minutes—or centrifuge at high speed until two successive spinnings give the same reading

## Procedures for the Sickle Cell Examination

If the oxygen is removed from the red cells of a healthy person the red cells do not lose their normal shape However if the oxygen is removed from the red cells of a person with sickle cell anemia the red cells become sickle shaped This is the principle of the sickle cell examination The sickling is apparently caused by some mechanical defect in the make-up of the red cells

In the bisulfite method of Daland and Castle the oxygen is removed by a reducing agent And in the wet preparation method of Scriver and Waugh the oxygen tension is lowered by stopping the flow of blood The blood is then removed from the patient and sealed from the air The two procedures follow

### Bisulfite Method (Daland and Castle)

1 Prepare the bisulfite reagent as follows Using the rough balance weigh out 0 5 gram of sodium metabisulfite ($Na_2S_2O$) Dissolve in 25 milliliters of distilled water This reagent must be prepared fresh (For convenience 200 milligram tablets may be purchased from Eli Lilly Co Indianapolis Indiana The solution may then be made by simply dissolving 1 tablet in 10 cc of distilled water )
2 Make a finger puncture and place 1 drop of blood on a glass slide
3 Add 1 drop of the bisulfite reagent and mix with an applicator stick
4 Place a cover glass on the preparation and gently press to spread out the cells
5 Set aside for 15 minutes
6 Examine with the oil immersion objective of the microscope Sickle shaped red cells are illustrated in Figure 44
7 If sickle shaped red cells are present report the test as positive If not report the test as negative

*Normal Values*

| | |
|---|---|
| Men | 40–50% |
| Women | 37–47% |

## SICKLE CELL EXAMINATION

**Information Significant to the Student**

*The sickle cell examination is an inspection of the red cells* for sickle shaped forms (Fig 44) They are present in sickle cell anemia a disease confined mainly to members of the Negro race The disease has an active and a passive stage

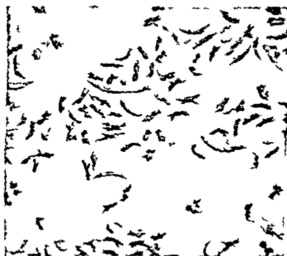


Fig 44 —Sickle shaped red cells (Wet preparation from Haden *Principles of Hematology* )

In the active stage the sickle shaped cells can be seen on a blood slide which has been stained with Wright s stain In the passive stage however they are rarely found on a blood slide but can be readily demonstrated in special preparations

3 While the rubber band is still on the finger make a finger puncture and place a drop of blood on the slide
4 On the fleshy portion of the palm of your hand just below the little finger rub some petroleum jelly
5 Rim the edges of a coverslip by scraping the petroleum jelly off your hand
6 Place the coverslip over the blood so that the drop is sealed under the coverslip
7 Using the oil immersion objective examine the preparation for sickle shaped red cells (Fig 44) If no sickling is immediately present put the slide aside and examine again at the end of 1 6 12 and 24 hours Sometimes the reaction is delayed and therefore do not report the test as negative until after the 24 hour inspection

## FRAGILITY TEST

### Information Significant to the Student

When blood is placed in an 0 85% salt solution the red cells neither swell nor shrink However if the salt solution is less than 0 85% (a hypotonic solution) water enters the cells by a process known as osmosis The cells swell and eventually rupture or hemolyze The more fragile the cells the quicker the hemolysis In hemolytic jaundice, the fragility of the red cells is increased whereas in obstructive jaundice, the fragility is decreased Consequently this test is a considerable aid to the physician in the diagnosis of these two diseases

### Procedures for the Fragility Test

The fragility of the red cells is determined by placing them in a graded series of hypotonic salt solutions and noting the extent of hemolysis after a period of two hours Either Sanford s method or a modified Sanford method may be used

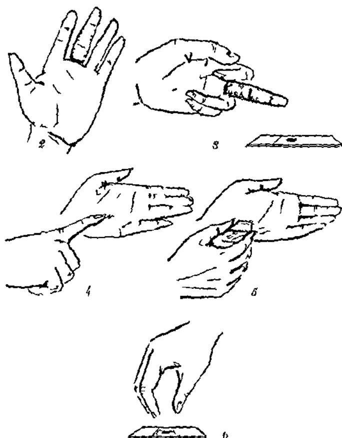


Fig 45 — Wet preparation method for sickle cell examination

**Wet Preparation Method (Scriver and Waugh)**

1. Get materials for a finger puncture a few coverslips a glass slide a rubber band and some petroleum jelly
2. Place the rubber band around the patient s finger in such a tight manner that it will stop the circulation Leave it there for about 5 minutes

tion in any test tube may be obtained by multiplying the test tube number by 0.02 For example test tube number 25 has a salt solution of $25 \times 0.02 = 0.50\%$

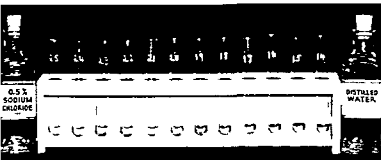



FIG 46 —Set up for a fragility test (Giffen and Sanford)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number on test tube | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 |
| Number drops 0.5% NaCl | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 |
| Number drops dist water | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| % salt sol obtained | 50 | 48 | 46 | 44 | 42 | 40 | 38 | 36 | 34 | 32 | 30 | 28 |

4 Take the patient's rack and materials for a venipuncture to the patient Make a venipuncture and withdraw a few cc of blood Leave the needle on the syringe and add one drop of blood to each tube Mix

5 In order to get blood for the control rack make a venipuncture on a normal person and withdraw a few cc of blood Leave the needle on the syringe and add one drop to each tube of the control rack Mix

to make the determination Both procedures are equally satisfactory The modified Sanford method simply uses larger quantities and thus sharpens the readings The procedures are given below

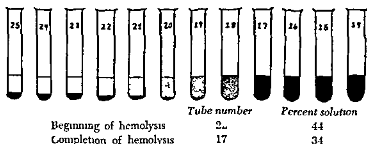
There are various modifications to the following tests In addition to the series of salt concentrations given in the procedure higher concentrations of salt may be used It is also possible although not necessary to make the final readings in a colorimeter

If increased fragility is suspected some laboratories run a simple screening test before setting up the fragility test The screening test consists of adding a few drops of the patient s blood to 5 cc of 0 5% sodium chloride This is gently mixed and centrifuged If the supernatant fluid is colorless increased fragility is ruled out However if the supernatant fluid shows a faint pink tinge increased fragility is indicated and further quantitative tests are run

**Sanford Method**

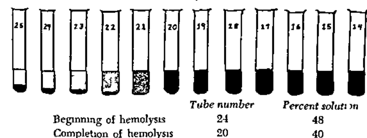
1 Dry some chemically pure sodium chloride in an oven and make an accurate 0 5% solution as follows Using the analytical balance weigh out 0 50 grams of the salt Place in a 100 cc volumetric flask and add distilled water to the 100 cc mark Mix
2 Get 2 test tube racks Label one *Control* and the other *Patient* Place 12 small test tubes in each rack and label the tubes in each rack as follows 25 24 23 22 21 20 19 18 17 16 15 and 14 (See Fig 46 )
3 Using the chart below add to the test tubes of each rack the number of drops of 0 5% sodium chloride and distilled water indicated Thus the test tubes labeled 25 get 25 drops of the 0 5% sodium chloride solution and 0 0 drops of distilled water The per cent salt solution obtained by this dilution process is given in the bottom row of the chart Note that the per cent salt solu

NORMAL BLOOD



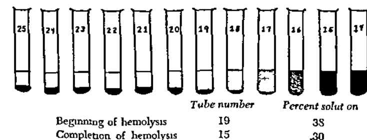

| | *Tube number* | *Percent solution* |
|---|---|---|
| Beginning of hemolysis | 22 | 44 |
| Completion of hemolysis | 17 | 34 |

HEMOLYTIC JAUNDICE




| | *Tube number* | *Percent solution* |
|---|---|---|
| Beginning of hemolysis | 24 | 48 |
| Completion of hemolysis | 20 | 40 |

*OBSTRUCTIVE JAUNDICE*




| | *Tube number* | *Percent solution* |
|---|---|---|
| Beginning of hemolysis | 19 | 38 |
| Completion of hemolysis | 15 | 30 |

FIG 47 —Normal and abnormal results of the fragility test

6 Let both racks stand at room temperature for 2 hours
7 Take the control rack and starting with tube number 25 and going to the right toward the lower numbered tubes note the test tube which contains the first faint pink tinge This is the beginning of hemolysis (Fig 47) Record the test tube number and the per cent salt solution Continue going to the right toward the lower numbered tubes and note the first tube which contains no sediment of cells This is the completion of hemolysis (Fig 47) Record the test tube number and the per cent salt solution Now take the patient's rack and in the same manner record the readings for the beginning and completion of hemolysis
8 Report the beginning and completion of hemolysis for both the control and the patient

*Normal Values*

| | *Tube Number* | *Per Cent Solution* |
|---|---|---|
| Beginning of hemolysis | 22 | 44 |
| Completion of hemolysis | 17 | 34 |

**Modified Sanford Method**

1 Dry some chemically pure sodium chloride in an oven Make an accurate 0 5% solution as follows Using the analytical balance weigh out 0 50 gram of the salt Place in a 100 cc volumetric flask and add distilled water to the 100 cc mark Mix
2 Get 2 test tube racks Label one *Control* and the other *Patient* Place 12 small test tubes in each rack and label the tubes in each rack as follows 25 24 23 22 21 20 19 18 17 16 15 and 14 (See Fig 46)

| Number on test tube | Number cc 0.5% NaCl | Number cc dist. water | % salt sol. obtained |
|---|---|---|---|
| 25 | 2.5 | 0.0 | 50 |
| 24 | 2.4 | 0.1 | 48 |
| 23 | 2.3 | 0.2 | 46 |
| 22 | 2.2 | 0.3 | 44 |
| 21 | 2.1 | 0.4 | 42 |
| 20 | 2.0 | 0.5 | 40 |
| 19 | 1.9 | 0.6 | 38 |
| 18 | 1.8 | 0.7 | 36 |
| 17 | 1.7 | 0.8 | 34 |
| 16 | 1.6 | 0.9 | 32 |
| 15 | 1.5 | 1.0 | 30 |
| 14 | 1.4 | 1.1 | 28 |

3. Using the chart below add to the test tubes of each rack the amount of 0.5% sodium chloride and distilled water indicated. Thus the test tubes labeled 25 get 2.5 cc. of the 0.5% sodium chloride and 0.0 cc. of distilled water. The per cent salt solution obtained by this dilution process is given in the bottom row of the chart. Note that the per cent salt solution in any test tube may be obtained by multiplying the test tube number by 0.02. For example, test tube number 25 has a salt solution of 25 × 0.02 = 0.50%.
4. Take the patient's rack and materials for a venipuncture to the patient. Make a venipuncture, withdraw a few cc. of blood and add 2 drops to each tube. Mix.
5. Make a venipuncture on a normal person, withdraw a few cc. of blood and add 2 drops to each tube of the control rack. Mix.
6. Let both racks stand at room temperature for 2 hours.
7. Take the control rack and, starting with tube 25 and going to the right toward the lower numbered tubes, note the test tube which contains the first faint pink tinge. This is the beginning of hemolysis (Fig. 47). Record the test tube number and the per cent salt solution. Continue going to the right toward the lower numbered tubes and note the first tube which contains no sediment of cells. This is the completion of hemolysis (Fig. 47). Record the test tube number and the per cent salt solution. Now take the patient's rack and in the same manner record the reading for the beginning and completion of hemolysis.
8. Report the beginning and completion of hemolysis for both the control and the patient.

In the following procedures a new methylene blue solution may be used in place of the brilliant cresyl blue solution. Its preparation is given in the Appendix on page 240 It is also permissible to use oxalated blood in place of finger tip blood If the oxalated blood is not used immediately however it should be stored in the refrigerator

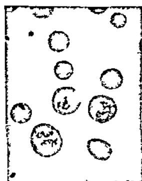


FIG 48 —Reticulocytes stained with a vital stain (Haden *Principles of Hematology* )

**Dry Method**

1 Get materials for a finger puncture Into a small test tube place 3 drops of a 1% normal saline solution of brilliant cresyl blue (preparation given in Appendix on page 239)
2 Make a finger puncture and add 3 drops of blood to the test tube containing the brilliant cresyl blue Mix Let stand several minutes in order to stain the cells
3 Gently shake the mixture of blood and brilliant cresyl blue place a small drop on a glass slide and make a blood slide
4 Allow to dry and counter stain with Wright s stain.

*Normal Values*

| | Tube Number | Per Cent Solution |
|---|---|---|
| Beginning of hemolysis | 22 | 44 |
| Completion of hemolysis | 17 | 34 |

## RETICULOCYTE COUNT

### Information Significant to the Student

There are 6 different stages in the development of a red cell The first 4 stages are the young cells which are present in the cell factories of the bone marrow The latter 2 stages are the older cells—the reticulocytes and erythrocytes—which are present in the blood stream Under normal conditions there are 1 to 2 reticulocytes for every 100 erythrocytes This gives us a normal reticulocyte count of 1 to 2%

In certain anemias however there is a strike in the cell factories of the bone marrow The production of red cells is decreased and the reticulocyte count drops If the strike can be ended—by means of medication—the count returns to normal

The reticulocyte count thus acts as a messenger of the bone marrow A drop in the count signaling a strike and a rise in the count indicating recovery This information is extremely useful in the diagnosis and treatment of many types of anemia

### Procedures for the Reticulocyte Count

In order to stain the granular net like substance in the reticulocyte (Fig 48) a special dye must be used and the cells stained while the blood is fresh Such a stain is called a vital stain The count is then made by calculating the percentage of reticulocytes found in the company of erythrocytes (mature red cells)

For the reticulocyte count the dry method and the wet method are used Both are equally satisfactory The procedures are given below

5 Let stand 10 minutes in order to stain the cell
6 Using the oil immersion objective record the number of reticulocytes (Fig 48) seen while counting 1 000 mature red cells (erythrocytes) In order to narrow the microscopic field and thus make it easier to count the cells some technicians place a piece of paper containing a window in the eye piece of the microscope Thus



7 Calculate the percentage For example if 10 reticulocytes were seen the percentage is found as follows

$$\frac{10 \text{ (reticulocytes)}}{1\,000 \text{ (erythrocytes)}} \times 100 = 1\%$$

*Normal Values*

| | |
|---|---|
| Adults | 1% to 2% |
| Infants | 4% to 8% |

## RED CELL INDICES

The red cell indices furnish the physician with information regarding the volume and hemoglobin content of the patient s red cells This information is useful in the diagnosis and treatment of many types of anemia Although the calculation of the indices are not met routinely by the technician they are frequently found on student examinations

Before the red cell indices are considered the student should refresh his memory regarding mathematical shorthand and equivalents of weights and measures The following information will be used in the discussion

5 Using the oil immersion objective record the number of reticulocytes (Fig 48) seen while counting 1 000 mature red cells (erythrocytes) In order to narrow the microscopic field and thus make it easier to count the cells some technicians place a piece of paper containing a window in the eye piece of the microscope Thus



6 Calculate the per cent For example if 10 reticulocytes were seen the percentage is found as follows

$$\frac{10 \text{ (reticulocytes)}}{1\,000 \text{ (erythrocytes)}} \times 100 = 1\%$$

*Normal Values*

| | |
|---|---|
| Adults | 1% to 2% |
| Infants | 4% to 8% |

**Wet Method**

1 Get a bottle of a 1% methyl alcohol solution of brilliant cresyl blue (preparation given in the Appendix on page 239)
2 Place a drop of the brilliant cresyl blue on a glass slide With the same technique used in making a blood smear spread the brilliant cresyl blue over the slide
3 Get a few coverslips and materials for a finger puncture
4 Make a finger puncture place a drop of blood on the stained slide and cover with a coverslip (The coverslip may be rimmed with petrolatum to prevent drying )

*Equivalents of Weights and Measures*

The equivalents of weights and measures which will be referred to in this section are given below. A list of abbreviations is also included.

Abbreviations

| | | |
|---|---|---|
| Gm | = | gram |
| cm | = | centimeter |
| mm. | = | millimeter |
| cc | = | cubic centimeter |
| cu mm | = | cubic millimeter |

Equivalents

| | | |
|---|---|---|
| 1 Gm | = | 10 micromicrograms |
| 1 cm | = | 10 mm |
| 1 mm | = | 10 microns |
| 1 cc | = | $10^3$ cu mm. |
| 1 cu mm | = | 10 cu microns |

The red cell indices are outlined and discussed in the following manner:

- I Relationships between the hemoglobin content and the red cell count
  - A Mean corpuscular hemoglobin
  - B Color index
- II Relationships between the cell volume and the red cell count
  - A Mean corpuscular volume
  - B Volume index
- III Relationships between the hemoglobin content and the cell volume
  - A Mean corpuscular hemoglobin concentration
  - B Saturation index

## MEAN CORPUSCULAR HEMOGLOBIN

### (*Average Cell Hemoglobin*)

The mean corpuscular hemoglobin is the weight of hemoglobin in the average red cell. The normal values are 27 to 32 micromicrograms. When the patient's hemoglobin concen

*Mathematical Shorthand*

By the use of mathematical shorthand considerable time may be saved in the recognition multiplication and division of large numbers For example 1 000 000 may be written as $10^6$ And 1 000 000 multiplied by 1 000 000 then becomes simply $10^6 \times 10^6$ And 1 000 000 divided by 1 000 000 then becomes simply $\frac{10^6}{10^6}$

The recognition multiplication and division of mathematical shorthand which will be used in this discussion are given below

Recognition

$10^1 = 10$
$10 = 100$
$10^3 = 1\,000$
$10^4 = 10\,000$
$10^5 = 100\,000$
$10^6 = 1\,000\,000$
$10^{12} = 1\,000\,000\,000\,000$
$2 \times 10^2 = 2 \times 100 = 200$
$2 \times 10^3 = 2 \times 1\,000 = 2\,000$

Multiplication

When 10 is multiplied by $10^b$ (a and b stand for any number) the rule is Add the exponents Thus

$$10 \times 10^b = 10^{+b}$$

For example $10^2 \times 10^3 = 10^3 = 10^5$
and $10 \times 10^6 = 10^{+6} = 10^2$

*Division*

When 10 is divided by $10^b$ the rule is Subtract the exponents

Thu $\frac{10^a}{10^b} = 10^{-b}$

For example $\frac{10^2}{10^5} = 10^{2-5} = 10^7$

and $\frac{10^9}{10^6} = 10^{-6} = 10$

$$\frac{14 \text{ Gm}}{100 \text{ cc}} \times \frac{10^{12}}{10} = \frac{14 \times 10^{12} \text{ micromicrograms}}{10^5 \text{ cu mm}}$$

By dividing the figures on the right hand side of the equal sign we have the micromicrograms of Hb in 1 cu mm of blood Thus

$$14 \times \frac{10^{12}}{10} = 14 \times 10 \text{ micromicrograms}$$

(3) Finding the denominator
Since the patient s red cell count is given as 5 000 000 ($5 \times 10^6$) cells per cu mm the denominator does not have to be found

(4) Substituting in the formula of step (1)

$$\text{Mean corpuscular Hb} = \frac{\text{Micromicrograms of Hb in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

$$= \frac{14 \times 10}{5 \times 10}$$

$$= \frac{14 \times 10}{5}$$

$$= 28 \text{ micromicrograms}$$

### Short Method

$$M\,C\,H = \frac{\text{Hb in grams per 100 cc} \times 10}{\text{RBC in million per cu mm}}$$

Example Patient s hemoglobin is 14 grams and RBC is 5 000 000

$$M\,C\,H = \frac{14 \times 10}{5\ 0}$$

$$= 28 \text{ micromicrogram}$$

tration and red cell count have been determined the problem is solved in the manner given below

The mean corpuscular hemoglobin is often abbreviated M C H There are two methods of finding the M C H long method and short method The long method gives all the steps involved whereas the short method consolidates several steps The procedures follow

## Long Method

*Given*

| | |
|---|---|
| Patient s hemoglobin | 14 Gm per 100 cc blood |
| Patient s red cell count | 5 000 000 cells per cu mm blood |

The above hemoglobin may be written as $\frac{14\ \text{Gm}}{100\ \text{cc}}$

and the red cell count may be written as $5 \times 10$

*Problem*

Find the mean corpuscular hemoglobin

*Solution*

(1) Formula

$$\text{Mean corpuscular Hb} = \frac{\text{Micromicrograms of Hb in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

(2) Finding the numerator

The problem here is to convert the patient s 14 grams of hemoglobin per 100 cc to micromicrograms per cu mm It is done as follows

From the table of weights and measures it is known that

1 Gm = $10^{2}$ micromicrograms
1 cc = 10 cu. mm

With this information the patient s 14 Gm of hemoglobin is converted to micromicrograms and the 100 cc of blood is converted to cu mm Thus

$$Color\ index = \frac{\dfrac{\text{Patient s hemoglobin}}{\text{Normal hemoglobin}}}{\dfrac{\text{Patient s red cell count}}{\text{Normal red cell count}}}$$

$$= \frac{0\ 76}{0\ 80} = 0\ 95$$

### Short Method

$$C I = \frac{\text{Hb in \%}}{\text{First 2 figures of RBC} \times 2}$$

Example Patient s hemoglobin is 100% and RBC is 5 000 000

$$C I = \frac{100}{50 \times 2} = 1\ 0$$

## MEAN CORPUSCULAR VOLUME

### (*Average Cell Volume*)

The mean corpuscular volume is the volume occupied by the average red cell The normal values are 80 to 90 cubic microns When the patient s hematocrit reading (per cent of red cells in a given volume of blood) and red cell count have been determined the problem is solved in the manner given below

The mean corpuscular volume is usually abbreviated M C V There are 2 methods of finding the M C V long method and short method The long method gives all the steps involved whereas the short method consolidates several steps The procedures follow

### Long Method

*Given*

| | |
|---|---|
| Patient s hematocrit reading | 40% |
| Patient s red cell count | 5 000 000 cells per cu mm blood |

The above hematocrit reading may be written as 40 and the red cell count may be written as $5 \times 10^6$

## COLOR INDEX

In order to determine this index hematologists have designated 14 5 Gm as the normal value for the hemoglobin concentration and 5 000 000 as the normal value for the red cell count When the patient s hemoglobin and red cell count are compared with these normal values the results can be expressed as a ratio which is known as the color index The normal range is between 0 90 and 1 10

The color index is usually abbreviated C I There are two methods of finding the C I long method and short method The long method gives all the steps involved whereas the short method consolidates several steps The procedures follow

### Long Method

*Given*

| | Patient | Normal |
|---|---|---|
| Gm of hemoglobin | 11 0 | 14 5 |
| Red cell count | 4 000 000 | 5 000 000 |

*Problem*

Find the color index

*Solution*

(1) Formula

$$\text{Color index} = \frac{\dfrac{\text{Patient s hemoglobin}}{\text{Normal hemoglobin}}}{\dfrac{\text{Patient s red cell count}}{\text{Normal red cell count}}}$$

(2) Finding the numerator

$$\frac{\text{Patient s hemoglobin}}{\text{Normal hemoglobin}} = \frac{11\ 0}{14\ 5} = 0\ 76$$

(3) Finding the denominator

$$\frac{\text{Patient s red cell count}}{\text{Normal red cell count}} = \frac{4\ 000\ 000}{5\ 000\ 000} = 0\ 80$$

(4) Substituting in the formula of step (1)

$$\text{Color index} = \frac{\dfrac{\text{Patient's hemoglobin}}{\text{Normal hemoglobin}}}{\dfrac{\text{Patient's red cell count}}{\text{Normal red cell count}}}$$

$$= \frac{0.76}{0.80} = 0.95$$

**Short Method**

$$\text{C.I.} = \frac{\text{Hb in \%}}{\text{First 2 figures of RBC} \times 2}$$

Example: Patient's hemoglobin is 100% and RBC is 5,000,000.

$$\text{C.I.} = \frac{100}{50 \times 2} = 1.0$$

## MEAN CORPUSCULAR VOLUME

### *(Average Cell Volume)*

The mean corpuscular volume is the volume occupied by the average red cell. The normal values are 80 to 90 cubic microns. When the patient's hematocrit reading (per cent of red cells in a given volume of blood) and red cell count have been determined, the problem is solved in the manner given below.

The mean corpuscular volume is usually abbreviated M.C.V. There are 2 methods of finding the M.C.V.: long method and short method. The long method gives all the steps involved whereas the short method consolidates several steps. The procedures follow.

**Long Method**

*Given*

| | |
|---|---|
| Patient's hematocrit reading | 40% |
| Patient's red cell count | 5,000,000 cells per cu. mm. blood |

The above hematocrit reading may be written as .40 and the red cell count may be written as $5 \times 10^6$.

*Problem*

Find the mean corpuscular volume

*Solution*

(1) Formula

$$\text{Mean corpuscular volume} = \frac{\text{Cubic microns of cells in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

(2) Finding the numerator

The hematocrit reading is the per cent of red cells in a given volume of blood. Therefore the volume of red cells in 1 cu mm of patient's blood is found as follows

| Vol of blood | × | Hematocrit reading | = | Vol of cells |
|---|---|---|---|---|
| 1 cu mm | × | 40 | = | 40 cu mm |

To convert the 40 cu mm of cells to cu microns multiply by $10^9$ (the number of cu microns in 1 cu mm). Thus

40 cu mm × 10 = cu microns

Therefore

$40 \times 10^9$ = cu microns of cells in 1 cu mm of blood

(3) Finding the denominator

Since the patient's red cell count is given as 5,000,000 ($5 \times 10^6$) cells per cu mm, the denominator does not have to be found

(4) Substituting in the formula of step (1)

$$\text{Mean corpuscular volume} = \frac{\text{Cubic microns of cells in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

$$= \frac{40 \times 10}{5 \times 10^6}$$

$$= \frac{40 \times 10}{5}$$

= 80 cu microns

**Short Method**

$$MCV = \frac{\text{Hematocrit reading} \times 10}{\text{RBC in millions per cu mm}}$$

Example Patient s hematocrit reading is 40% and RBC is 5 000 000

$$MCV = \frac{40 \times 10}{5\ 0} = 80 \text{ cu microns}$$

## VOLUME INDEX

In order to determine this index hematologists have designated 42% as the normal value for the hematocrit reading and 5 000 000 as the normal value for the red cell count When the patient s hematocrit reading and red cell count are compared with these normal values the results can be expressed as a ratio which is known as the volume index It is found in the manner given below The normal range is between 0 90 and 1 10

The volume index is usually abbreviated V I There are two methods of finding the V I long method and short method The long method gives all the steps involved whereas the short method consolidates several steps The procedures follow

**Long Method**

*Given*

| | *Patient* | *Normal* |
|---|---|---|
| Hematocrit reading | 38% | 42% |
| Red cell count | 4 500 000 | 5 000 000 |

*Problem*

Find the volume index

*Solution*

(1) Formula

$$\text{Volume index} = \frac{\dfrac{\text{Patient s hematocrit reading}}{\text{Normal hematocrit reading}}}{\dfrac{\text{Patient s red cell count}}{\text{Normal red cell count}}}$$

*Problem*

Find the mean corpuscular volume

*Solution*

(1) Formula

$$\text{Mean corpuscular volume} = \frac{\text{Cubic microns of cells in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

(2) Finding the numerator

The hematocrit reading is the per cent of red cells in a given volume of blood Therefore the volume of red cells in 1 cu mm of patient s blood is found as follows

| Vol of blood | $\times$ Hematocrit reading | = Vol of cell |
|---|---|---|
| 1 cu mm | $\times$ 40 | = 40 cu mm |

To convert the 40 cu mm of cells to cu microns multiply by $10^9$ (the number of cu microns in 1 cu mm ) Thus

40 cu mm $\times$ 10 = cu microns

Therefore

$40 \times 10^9$ = cu microns of cell in 1 cu mm of blood

(3) Finding the denominator

Since the patient s red cell count is given as 5 000 000 ($5 \times 10^6$) cells per cu mm the denominator does not have to be found

(4) Substituting in the formula of step (1)

$$\text{Mean corpuscular volume} = \frac{\text{Cubic microns of cells in 1 cu mm of blood}}{\text{Number of cells in 1 cu mm of blood}}$$

$$= \frac{40 \times 10}{5 \times 10}$$

$$= \frac{40 \times 10}{5}$$

= 80 cu microns

it is in common usage it will be included here When the patient s hemoglobin concentration and hematocrit reading have been determined the problem is solved in the manner given below The normal values for the mean corpuscular *hemoglobin concentration are 33 to 38%.*

The mean corpuscular hemoglobin concentration is usually abbreviated M C H C There are two methods of finding the M C H C long method and short method The long method gives all the steps involved whereas the short method consolidates several steps The procedures follow

### Long Method

*Given*

| | |
|---|---|
| Patient s hemoglobin | 14 Gm. per 100 cc blood |
| Patient s hematocrit reading | 40% |

*Problem*

Find the mean corpuscular hemoglobin concentration.

*Solution*

(1) Formula

$$\text{Mean corpuscular Hb conc} = \frac{\text{Gm. of Hb in 100 cc blood}}{\text{cc of packed red cells in 100 cc blood}} \times 100$$

(2) Finding the numerator

Since the patient s hemoglobin is given as 14 Gm per 100 cc of blood the numerator does not have to be found

(3) *Finding the denominator*

Since the hematocrit reading is the per cent of red cells in a given volume of blood the cc of packed red cells in 100 cc of blood is found as follows

Vol of blood × Hematocrit reading = Vol packed red cell

100 cc × 40% = 40 cc

(2) Finding the numerator

$$\frac{\text{Patient's hematocrit reading}}{\text{Normal hematocrit reading}} = \frac{38}{42} = 90$$

(3) Finding the denominator

$$\frac{\text{Patient's red cell count}}{\text{Normal red cell count}} = \frac{4\,500\,000}{5\,000\,000} = 90$$

(4) Substituting in the formula of step (1)

$$\text{Volume index} = \frac{\dfrac{\textit{Patient's hematocrit reading}}{\textit{Normal hematocrit reading}}}{\dfrac{\text{Patient's red cell count}}{\textit{Normal red cell count}}}$$

$$= \frac{90}{90} = 1\ 0$$

**Short Method**

$$\text{V I} = \frac{\text{Hematocrit reading} \times 0\ 118}{\text{RBC } \textit{in millions per cu mm}}$$

Example Patient s hematocrit reading is 38% and RBC is 4 500 000

$$\text{V I} = \frac{38 \times 0\ 118}{4\ 5}$$

$$= 1\ 0$$

## MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION

The per cent of hemoglobin in the patient s packed red cell volume is referred to as the mean corpuscular hemoglobin concentration Actually the term is a misnomer * but since

* From a mathematical point of view whenever the word average or mean is found in a formula the number of particles used to arrive at the average must be included in the denominator This does not occur in this formula Actually what the formula does is to find the grams of hemoglobin per cc of packed red cells This is then multiplied by 100 and the per cent obtained

*Problem*

Find the saturation index.

*Solution*

(1) Formula

$$\text{Saturation index} = \frac{\dfrac{\text{Patient's hemoglobin}}{\text{Normal hemoglobin}}}{\dfrac{\text{Patient's hematocrit}}{\text{Normal hematocrit}}}$$

(2) Finding the numerator

$$\frac{\text{Patient's hemoglobin}}{\text{Normal hemoglobin}} = \frac{12.0}{14.5} = 0.83$$

(3) Finding the denominator

$$\frac{\text{Patient's hematocrit}}{\text{Normal hematocrit}} = \frac{36}{42} = 0.86$$

(4) Substituting in the formula of step (1)

$$\text{Saturation index} = \frac{\dfrac{\text{Patient's hemoglobin}}{\text{Normal hemoglobin}}}{\dfrac{\text{Patient's hematocrit}}{\text{Normal hematocrit}}}$$

$$= \frac{0.83}{0.86} = 0.97$$

**Short Method**

$$\text{S.I.} = \frac{\text{Hb in per cent}}{\text{Hematocrit reading} \times 2.3}$$

Example: Patient's Hb is 80% and hematocrit reading is 36%.

$$\text{S.I.} = \frac{80}{36 \times 2.3} = 0.97$$

(4) Substituting in the formula of step (1)

$$\text{Mean corpuscular Hb conc.} = \frac{\text{Gm of Hb in 100 cc blood}}{\text{cc of packed red cells in 100 cc blood}} \times 100$$

$$= \frac{14}{40} \times 100$$

$$= 35\%$$

### Short Method

$$\text{M.C.H.C.} = \frac{\text{Hb in grams per 100 cc} \times 100}{\text{Hematocrit reading}}$$

Example: Patient's Hb is 14 grams and hematocrit reading is 40%.

$$\text{M.C.H.C.} = \frac{14 \times 100}{40} = 35\%$$

## SATURATION INDEX

In order to determine this index, hematologists have designated 14.5 Gm. as the normal value for the hemoglobin concentration and 42% as the normal value for the hematocrit reading. (These are the same values used for the determination of the color index and volume index.) When the patient's hemoglobin and hematocrit reading are compared with these normal values, the results can be expressed as a ratio which is known as the saturation index. The normal range for the saturation index is 0.90 to 1.10.

The saturation index is usually abbreviated S.I. There are two methods of finding the S.I.: long method and short method. The long method gives all the steps involved whereas the short method consolidates several steps. The procedures follow.

### Long Method

*Given*

| | *Patient* | *Normal* |
|---|---|---|
| Gm of hemoglobin | 12.0 | 14.5 |
| Hematocrit reading | 36% | 42% |

This chapter presents those tests which are associated with blood coagulation The information which they render the physician is generally used in the diagnosis and treatment of hemorrhagic diseases The tests will be considered in the following order

Bleeding time
Coagulation time
Clot retraction time
Prothrombin time
Platelet count
Capillary resistance test

## BLEEDING TIME

**Information Significant to the Student**

The bleeding time is the time required for a small cut to stop bleeding The normal time is 1 to 3 minutes It is increased in the following two hemorrhagic diseases essential thrombocytopenic purpura and hemorrhagic disease of the newborn

**Procedures for the Bleeding Time**

The bleeding time is determined by making a small cut in the skin and noting the time required for bleeding to cease The procedures for the Duke method and the Ivy method follow

**Duke Method**

1 Get materials for a finger puncture a piece of filter paper and a watch with a second hand
2 Puncture the finger deeply enough to insure a free flow of blood Record the time
3 At half minute intervals remove the drops of blood with the filter paper When blood ceases to flow record the time

Chapter 4

# Test Associated with Blood Coagulation

ALTHOUGH the exact mechanism of blood coagulation is not thoroughly understood the theory of Howell offers a workable hypothesis According to this theory the following five substances are involved in blood coagulation

| | | |
|---|---|---|
| 1 | Prothrombin | found in plasma |
| 2 | Antiprothrombin | found in plasma |
| 3 | Calcium | found in plasma |
| 4 | Fibrinogen | found in plasma |
| 5 | Thromboplastin | found in tissue juices and platelet disintegration |

In order for blood to clot the fifth constituent thromboplastin must be present It is obtained from tissue juices and the disintegration of platelets

When thromboplastin is present it neutralizes antiprothrombin and leaves prothrombin free The free prothrombin then reacts with calcium to form thrombin The thrombin *unites with fibrinogen to produce fibrin The fibrin* a net like substance entangles the blood cells to form the clot

The reaction may be thought of as taking place in the following manner

1 Prothrombin + Antiprothrombin *absence* of thromboplastin ⟶ bound prothrombin
2 Prothrombin + Antiprothrombin *presence* of thromboplastin ⟶ free prothrombin
3 Free prothrombin + Calcium ⟶ Thrombin
4 Thrombin + Fibrinogen ⟶ Fibrin
5 Fibrin + Blood cells ⟶ Clot

## COAGULATION TIME

### Information Significant to the Student

The coagulation time is the number of minutes required for blood to coagulate. It is increased in hemophilia, obstructive jaundice, and hemorrhagic disease of the newborn. The various methods of determination and their normal values are given below.

| | Minutes |
|---|---|
| 1 Capillary blood method | |
| *a* Slide method | 2 to 6 |
| *b* Capillary tube method | 2 to 6 |
| 2 Venous blood methods | |
| *a* Lee and White method | 5 to 10 |
| *b* Howell method | 10 to 30 |

The capillary blood methods have a shorter coagulation time than the venous blood methods because substances (essentially thromboplastin) in the tissue juices aid in the coagulation of blood. Because of the difference in normal values, the technician should always mention the method of procedure and the normal values in his report.

### Procedures for the Coagulation Time

The coagulation time is determined by placing a small amount of blood on a glass slide (or in a tube) and recording the time required for coagulation to take place. The procedures for the methods listed above are given in detail.

#### Capillary Blood Methods

*A Slide Method*

1. Get materials for a finger puncture, a glass slide, a needle, and a watch with a second hand.
2. Make a finger puncture deep enough to insure a free flow of blood. Wipe away the first 2 drops, record the time, and place several separate drops on the slide.

4 The bleeding time is the time elapsed between the puncture of the finger and the cessation of bleeding (Fig 49)

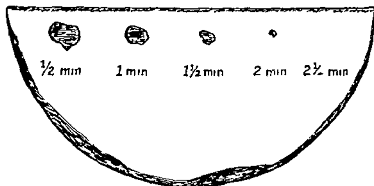



Fig 49 —Normal bleeding time

**Ivy Method**

1 Get a blood pressure kit materials for a finger puncture a piece of filter paper and a watch with a second hand
2 Place the blood pressure cuff just above the bend in the elbow and inflate to 40 mm pressure
3 Cleanse the surface of the forearm with the antiseptic Dry Make a rather deep puncture on the fleshy portion of the forearm just below the bend in the elbow Record the time
4 At half minute intervals remove the drops of blood with the filter paper When blood ceases to flow record the time
5 The bleeding time is the time elapsed between the puncture of the forearm and the cessation of bleeding

Normal values 1 to 3 minutes

3 After filling the capillary tubes allow 2 minutes to elapse Then carefully break off a piece of the tubing and look for a span of fibrin between the broken ends (Fig 50) Continue at half minute intervals until a span of fibrin is seen Record the time

4 The coagulation time is the time elapsed between the filling of the capillary tubes and the appearance of the span of fibrin

Normal values 2 to 6 minutes

#### Venous Blood Methods

1 *Lee and White Method*

1 Get a sterile needle sterile syringe and 3 small test tubes Rinse them all with a sterile normal saline solution (preparation given in the Appendix on page 240) Label the test tubes #1 #2 and #3 Get a watch with a second hand

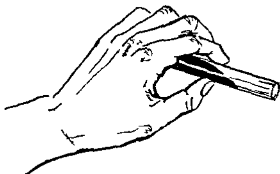


Fig 51 —Completion of the coagulation time in the venous blood methods

2 Make a venipuncture withdraw about 5 cc of blood record the time and transfer 1 cc of blood to each test tube

3 At half minute intervals draw the needle through a drop of blood until it picks up fibrin threads and drags them along Record the time

4 The time elapsed between the placing of the first drop of blood on the slide and the observance of the fibrin threads is the coagulation time

Normal values 2 to 6 minutes

*b Capillary Tube Method*

1 Get materials for a finger puncture several capillary tubes and a watch with a second hand

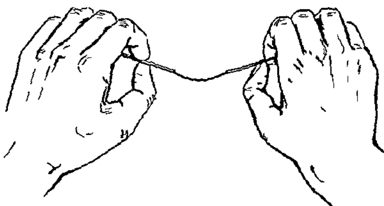


FIG 50 —Span of fibrin in the determination of the coagulation time (Capillary tube method )

2 Make a *finger puncture* deep enough to insure a free flow of blood Wipe away the first 2 drops record the time and fill 3 or 4 capillary tubes (Several capillary tubes are filled because they may be necessary if the coagulation time is prolonged )

# CLOT RETRACTION TIME

### Information Significant to the Student

The clot retraction time measures the ability of the blood clot to retract. In normal blood the clot retraction is well under way at the end of 2 hours and is complete at the end of 24 hours. The clot retraction time is increased whenever the platelet count is decreased. Consequently it is increased in the hemorrhagic disease essential thrombocytopenic purpura.

### Procedure for the Clot Retraction Time

The clot retraction time is determined by placing about 5 cc. of blood in a test tube and making several observations of the progress of clot retraction. The procedure follows:

1. Get materials for a venipuncture and a test tube capable of holding 5 cc. of blood.

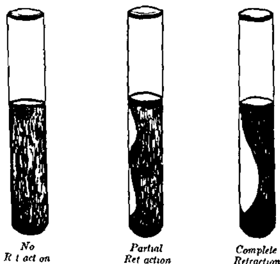



Fig. 52.—Retraction of the blood clot.

3. Allow 4 minutes to elapse
4. Now take tube #1 and gently tilt it every half minute until a clot forms and blood will not flow from the tube (Fig 51)
5. Now take tube #2 and gently tilt it every half minute until a clot forms and blood will not flow from the tube
6. Now take tube #3 and gently tilt it every half minute until a clot forms and blood will not flow from the tube. Record the time
7. The coagulation time is the time elapsed between the withdrawal of blood and the completion of coagulation in tube #3

   Normal values 5 to 10 minutes

*B Howell Method*

1. Take a small portion (about the size of your thumbnail) of petrolatum and dissolve it in about 8 cc of ether. Coat a syringe with the mixture by sucking it up into the syringe and then expelling it. Get a small test tube and sterile needle
2. Using the same syringe make a venipuncture and withdraw about 3 cc of blood. Record the time and transfer the blood to the test tube
3. Coagulation is complete when the blood doesn't flow from the tube (Fig 51). Therefore at half minute intervals gently tilt the tube until coagulation takes place. Record the time
4. The coagulation time is the time elapsed between the withdrawal of blood and the completion of coagulation

   Normal values 10 to 20 minutes

and thromboplastin This brings together all the necessary ingredients for the formation of a clot The time interval between the addition of the plasma and the appearance of the clot is called the prothrombin time

The method using Solu Plastin and the method using Simplastin are given below

**Method Using Solu Plastin**

1 Get materials for a venipuncture Into a test tube pipet exactly 0 5 cc of 1 34% (0 1 molar) sodium oxalate The preparation of the sodium oxalate is given in the Appendix on page 242
2 Make a venipuncture and add exactly 4 5 cc of blood to the tube Put the cork in the mouth of the tube and invert several times to mix the blood and anticoagulant (In order to measure out exactly 4 5 cc of blood some technicians withdraw about 5 cc remove the needle place several gauze pads over the neck of the syringe and force out blood until it reaches the 4 5 mark on the syringe The syringe then contains exactly 4 5 cc of blood )
3 Within the next 10 minutes centrifuge the blood
4 Carefully pipet off the plasma transfer to a test tube and place in a 37 C water bath NOTE If the plasma is not used immediately it should be stored in the refrigerator Upon removal from the refrigerator it should be warmed to 37 C
5 Pipet 0 1 cc of Solu Plastin into a small test tube Add 0 1 cc of the 0 0125 molar calcium chloride which comes with the Solu Plastin Mix Place the tube in the 37 C water bath
6 Note the time quickly add 0 1 cc of plasma mix and tilt the tube every few seconds

2 Make a venipuncture withdraw about 5 cc of blood, and transfer to the test tube
3 Place in an incubator at 37° C and observe at the end of 2 * 6 18 and 24 hours Each time record the clot retraction as no retraction partial retraction or complete retraction (Fig 52)

## PROTHROMBIN TIME

### Information Significant to the Student

During many operations the surgeon seeks to prevent blood clots And in various heart conditions the physician endeavors to dissolve blood clots Blood clots may be prevented and dissolved by the administration of Dicumarol ✓

The Dicumarol decreases the concentration of prothrombin *When prothrombin is decreased the blood takes longer* to clot This decreased clotting activity is favorable to the prevention and breakdown of blood clots

The decreased clotting activity however must be controlled for if certain limits are exceeded a hemorrhage may occur Consequently a yardstick is needed to measure clotting activity This yardstick is the prothrombin time

*The normal values for the prothrombin time are 12 to 17* seconds During treatment with Dicumarol the prothrombin time is usually kept between 28 and 40 seconds This is accomplished by regulating the dosage of the drug and running prothrombin times at frequent intervals

### Procedures for the Prothrombin Time

There are many procedures for determining prothrombin time Practically all are based on the original research of Dr A J Quick Basically the test involves adding plasma —which contains the prothrombin—to a solution of calcium

* Sometimes normal blood will form a clot which will stick to the walls of the test tube and any retraction of the clot will not be noticeable Therefore if the clot is sticking to the walls of the test tube at the end of two hours loosen it gently with a wooden applicator stick

mouth of the tube and invert several times to mix the blood and anticoagulant (In order to measure out exactly 4 5 cc of blood some technicians withdraw about 5 cc remove the needle place several gauze pads over the neck of the syringe and force out blood until it reaches the 4 5 mark on the syringe The syringe then contains exactly 4 5 cc of blood )

3 Within the next 10 minutes centrifuge the blood

4 Carefully pipet off the plasma transfer to a small test tube and place in a 37 C water bath NOTE If the plasma is not used immediately it should be stored in the refrigerator Upon removal from the refrigerator it should be warmed to 37 C

5 Preparation of Simplastin suspension If you have the *20 determination* vial of Simplastin add exactly 4 0 cc of distilled water and shake *gently* If you have the *6 determination* vial of Simplastin add 1 6 cc of distilled water and shake *gently* When not in use store in the refrigerator

6 Pipet 0 2 cc of the Simplastin suspension into a small test tube Place the tube in a 37 C water bath Let sit for a few minutes

7 Note the time quickly add 0 1 cc of plasma mix and tilt the tube every few seconds until a gelatinous clot appears Note the time (As an alternative procedure to tilting the tube some technicians use a nichrome wire loop and agitate the solution until the clot sticks to the loop )

8 The prothrombin time is the number of seconds elapsing between the addition of the plasma and the appearance of the gelatinous clot

until a gelatinous clot forms. Note the time. (As an alternative procedure to tilting the tube, some technicians agitate the mixture with a nichrome wire loop. When the clot forms, it sticks to the loop.)

7. The prothrombin time is the number of seconds elapsing between the addition of the plasma and the appearance of the gelatinous clot.
8. A control's time comes with the box of Solu-Plastin. In reporting the prothrombin time, some laboratories report the patient's time and the control's time. Other laboratories report not only the patient's time and control's time but also the prothrombin activity. This is discussed below.
9. The prothrombin activity is the patient's value compared with a control's value. For example, suppose the patient's plasma clotted in 20 seconds. And it had been found that a 50% solution of a control's plasma also clotted in 20 seconds. Then the patient's prothrombin activity would be 50%.
10. In order to compensate for differences in technique and reagents, each laboratory sets up its own graph or table for determining the prothrombin activity. The directions for preparing the graph are given with the bottle of Solu-Plastin.

**Method Using Simplastin**

1. Get materials for a venipuncture. Into a test tube, pipet exactly 0.5 cc of 1.34% (0.1 molar) sodium oxalate. The preparation of the sodium oxalate is given in the Appendix on page 242.
2. Make a venipuncture and add exactly 4.5 cc of blood to the tube. Put the cork in the

mouth of the tube and invert several times to mix the blood and anticoagulant. (In order to measure out exactly 4.5 cc. of blood, some technicians withdraw about 5 cc., remove the needle, place several gauze pads over the neck of the syringe and force out blood until it reaches the 4.5 mark on the syringe. The syringe then contains exactly 4.5 cc. of blood.)

3. Within the next 10 minutes, centrifuge the blood.
4. Carefully pipet off the plasma, transfer to a small test tube, and place in a 37° C. water bath. NOTE: If the plasma is not used immediately, it should be stored in the refrigerator. Upon removal from the refrigerator it should be warmed to 37° C.
5. Preparation of Simplastin suspension: If you have the *20 determination* vial of Simplastin, add exactly 4.0 cc. of distilled water and shake *gently*. If you have the *6 determination* vial of Simplastin, add 1.6 cc. of distilled water and shake *gently*. When not in use, store in the refrigerator.
6. Pipet 0.2 cc. of the Simplastin suspension into a small test tube. Place the tube in a 37° C. water bath. Let sit for a few minutes.
7. Note the time, quickly add 0.1 cc. of plasma, mix, and tilt the tube every few seconds until a gelatinous clot appears. Note the time. (As an alternative procedure to tilting the tube, some technicians use a nichrome wire loop and agitate the solution until the clot sticks to the loop.)
8. The prothrombin time is the number of seconds elapsing between the addition of the plasma and the appearance of the gelatinous clot.

until a gelatinous clot forms Note the time (As an alternative procedure to tilting the tube some technicians agitate the mixture with a nichrome wire loop When the clot forms it sticks to the loop )

7 The prothrombin time is the number of seconds elapsing between the addition of the plasma and the appearance of the gelatinous clot

8 A control s time comes with the box of Solu Plastin In reporting the prothrombin time some laboratories report the patient s time and the control s time Other laboratories report not only the patient s time and control s time but also the prothrombin activity This is discussed below

9 The prothrombin activity is the patient s value compared with a control s value For example suppose the patient s plasma clotted in 20 seconds And it had been found that a 50% solution of a control s plasma also clotted in 20 seconds Then the patient s prothrombin activity would be 50%

10 In order to compensate for differences in technique and reagents each laboratory sets up its own graph or table for determining the prothrombin activity The directions for preparing the graph are given with the bottle of Solu Plastin

#### Method Using Simplastin

1 Get materials for a venipuncture Into a test tube pipet exactly 0 5 cc of 1 34% (0 1 molar) sodium oxalate The preparation of the sodium oxalate is given in the Appendix on page 242

2 Make a venipuncture and add exactly 4 5 cc of blood to the tube Put the cork in the

| *1[illegible] second Control Column* | | *15 second Control Column* | | *16 second Control Column* | |
|---|---|---|---|---|---|
| *Patient's Prothrombin Time* | *Prothrombin Activity* | *Patient's Prothrombin Time* | *Prothrombin Activity* | *Patient's Prothrombin Time* | *Prothrombin Activity* |
| 14 sec | 100% | 15 sec | 100% | 16 sec | 100% |
| 15 sec | 86% | 16 sec | 86% | 17 sec | 86% |
| 16 sec | 72% | 17 sec | 72% | 18 sec | 75% |
| 17 sec | 60% | 18 sec | 61% | 19 sec | 63% |
| 18 sec | 49% | 19 sec | 50% | 20 sec | 50% |
| 19 sec | 44% | 20 sec | 45% | 21 sec | 46% |
| 20 sec | 40% | 21 sec | 41% | 22 sec | 39% |
| 21 sec | 35% | 22 sec | 35% | 23 sec | 36% |
| 22 sec | 28% | 23 sec | 34% | 24 sec | 34% |
| 23 sec | 2[illegible]% | 24 sec | 30% | 25 sec | 29% |
| 24 sec | 25% | 25 sec | 27% | 26 sec | 25% |
| 25 sec | 23% | 26 sec | 24% | 27 sec | 24% |
| 26 sec | 21% | 27 sec | 23% | 28 sec | 24% |
| 27 sec | 20% | 28 sec | 22% | 29 sec | 23% |
| 28 sec | 19% | 29 sec | 21% | 30 sec | 21% |
| 29 sec | 18% | 30 sec | 20% | 31 sec | 20% |
| 30 sec | 17% | 31 sec | 19% | 32 sec | 19% |
| 31 sec | 16% | 32 sec | 18% | 33 sec | 18% |
| 32 sec | 16% | 33 sec | 17% | 34 sec | 16% |
| 33 sec | 15% | 34 sec | 16% | 35 sec | 16% |
| 34 sec | 14% | 35 sec | 16% | 36 sec | 15% |
| 35 sec | 13% | 36 sec | 15% | 37 sec | 15% |
| 36 sec | 12.5% | 37 sec | 14% | 38 sec | 14% |
| *more than* 36 sec | *less than* 12.5% | 38 sec | 13% | 39 sec | 13% |
| | | 39 sec | 13% | 40 sec | 13% |
| | | 40 sec | 12.5% | 41 sec | 12.5% |
| | | *more than* 40 sec | *less than* 12.5% | *more than* 41 sec | *less than* 12.5% |

Fig 53 —Finding the prothrombin activity

## PLATELET COUNT

**Information Significant to the Student**

The four stages in the development of a blood platelet or thrombocyte are illustrated in Figure 54 The first three cells are found in the bone marrow only the final stage (the

9 A control s time is obtained by running a prothrombin time with normal or Diagnostic Plasma Some laboratories run a control with each test whereas other laboratories run a control with each new batch of Simplastin

10 In reporting the prothrombin time some laboratories report the patient s time and the control s time Other laboratories report not only the patient s time and the control s time but also the prothrombin activity The prothrombin activity is found in the manner indicated below

*How to Find the Prothrombin Activity*

The prothrombin activity is a comparison of the patient s prothrombin time to normal prothrombin time It is found as follows

Find the control s time by either referring to the box containing the reagent or running a prothrombin time with normal or Diagnostic Plasma The usual time for the control is 14 15 or 16 seconds

Find the column in Figure 53 which represents the control s time If the control s time is 14 seconds the column on the left is used If the control s time is 15 seconds the column in the middle is used And if the control s time is 16 seconds the column on the right is used

Refer to the correct column for your particular control get the patient s prothrombin time and read off the prothrombin activity

Example Suppose the control s time is 15 seconds We would then use the 15 second control column This is the middle column in Figure 53

Now suppose the patient s prothrombin time is 17 seconds By referring to this middle column we find that a patient s prothrombin time of 17 seconds gives a prothrombin activity of 72%

For the data appearing in Figure 53 we are indebted to a paper written by Dr Milton R Bronstein which appeared in the Journal of the Medical Society of New Jersey Vol 49 December 1952

**Procedure for a Platelet Count**

The direct method and indirect method are used to determine the platelet count In the direct method the blood is diluted placed in a counting chamber and the platelets are counted In the indirect method the ratio of platelets to red cells is determined by a study of the blood slide It is assumed that this ratio of platelets to red cells also exists in one cubic millimeter of blood Therefore the ratio is multiplied by the red cell count since the red cell count is the number of cells in 1 cu mm of blood The platelet count with this method is slightly higher than the results obtained by the direct method The two procedures follow

**Direct Method (Todd and Sanford)**

1 Get materials for a finger puncture a red cell pipet and a bottle of either Rees and Ecker or Leake and Guy diluting fluid These diluting fluids should be filtered before use They are prepared as indicated in the Appendix under Preparation of Solutions and Reagents
2 Moisten the bore of the red cell pipet by drawing diluting fluid up and down into the stem of the pipet (This keeps the platelets from sticking to the bore ) Make a finger puncture and wipe away the first drop Draw blood to the 0 5 mark and diluting fluid to the 101 mark This is a 1 in 200 dilution The remainder of the count should be completed within 15 minutes because the platelets disintegrate rapidly
3 Shake the pipet for 2 to 3 minutes discard the first 4 drops and charge a counting chamber Cover the counting chamber with a petri dish to decrease evaporation and let stand 5 minutes to allow the platelets to settle
4 Observe Figure 50 in which a circle has been placed around the platelets You will note

mature blood platelet) is present in the circulating blood When platelets disintegrate they release thromboplastin one of the essential factors in blood coagulation This is their primary function The platelet count in a normal person is 250 000 to 350 000 cells per cubic millimeter The count is markedly reduced in essential thrombocytopenic purpura—one of the hemorrhagic diseases

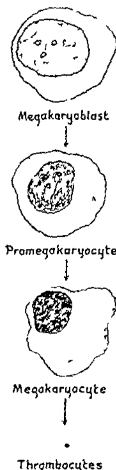



Fig 54 —Development of blood platelets (thrombocytes)

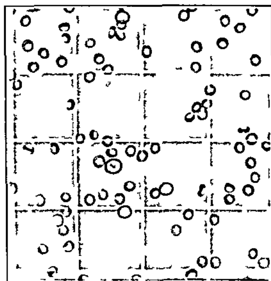


Fig 55 —Platelets seen with the high power (4 mm ) objective
Courtesy of Mr Cecil Gilliam )

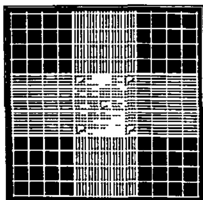


Fig 56 —Section of the counting chamber used in the platelet count
(Direct method )

that the platelets are about one tenth the size of red cells and sometimes come in groups of 2, 3, or 4. You will find that they appear as tiny glistening objects and are best seen when the fine adjustment of the microscope is rotated back and forth.

5. Using the high power (4 mm.) objective, count the platelets in the 5 sections marked with a "P" in Figure 56. (These are the same five sections used in making a red cell count.)
6. Calculate the number of platelets in 1 cubic millimeter of blood. For example, if a total of 30 platelets were counted in the 5 "P" sections, the calculation is made in the manner given below. The correction factors are the same as those used for the red cell count and they have been derived in the same manner.

| Total platelet in 5 "P" sect. | | Dil. cor. factor | | Vol. cor. factor | | Platelets in 1 cu. mm. |
|---|---|---|---|---|---|---|
| 30 | × | 200 | × | 50 | = | 300,000 |

Normal values: 250,000 to 350,000 per cu. mm.

#### Indirect Method

1. Get materials for a finger puncture, a few glass slides, a red cell pipet, and some red cell diluting fluid.
2. Make a finger puncture and wipe away the first drop. Dilute the blood for a red cell count by drawing blood to the 0.5 mark and diluting fluid to the 101 mark. Make a blood slide.
3. Stain the blood slide with Wright's stain; meanwhile make the red cell count.
4. Take the stained blood slide, use the oil immersion objective, and record the number of platelets seen while counting 1,000 red cells.

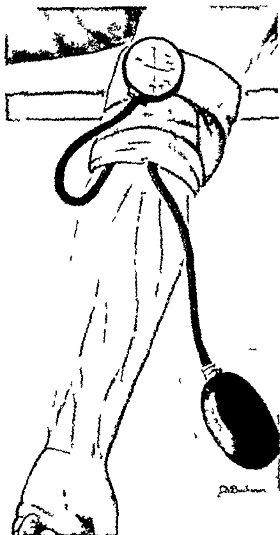


FIG 5 A positive capillary resistance test (From Kracke Roy R *Diseases of the Blood* 2nd edition J B Lippincott Co)

(Platelets of the blood slide are illustrated in Plate XII page 228. They are the six small bluish purple cells seen directly above the name Elise Tremaux.)

In order to narrow the microscopic field and thus make it easier to count the cells some technicians place a piece of paper containing a "window" in the eye piece of the microscope. Thus



5 Calculation The platelet count equals the ratio of platelets to red cells multiplied by the red cell count. For example if you have the following data the calculation is made in the manner given below

| | |
|---|---|
| Red cell count | 5 000 000 |
| Red cells counted on slide | 1 000 |
| Platelets recorded | 50 |

$$\text{Platelet count} = \frac{\text{Number of platelets counted on slide}}{\text{Number of red cells counted on slide}} \times \text{red cell count}$$

$$= \frac{50}{1\,000} \times 5\,000\,000$$

$$= 250\,000$$

Normal values 250 000 to 350 000 per cu mm

## CAPILLARY RESISTANCE TEST

### Information Significant to the Student

The capillary resistance test measures the ability of the capillaries to resist pressure. When a blood pressure cuff is placed above the bend in the elbow and inflated to about

Chapter 5

# Tests Related to Blood Groups

The discovery of blood groups opened new frontiers in the field of hematology. These frontiers have led to many new laboratory tests. Some tests are used to transfuse blood and other tests are used to aid infants in their battle against a ruthless hemolytic anemia. These tests are discussed in this chapter. The material is arranged as follows:

- Basic Theory
- ABO Grouping
- Rh Typing
- Cross Match
- Coombs Test
- Rh Titer

## BASIC THEORY

Blood may be separated into cells and plasma or serum. When the cells of one person are mixed with the plasma of another individual, the red cells often become clumped or agglutinated. The agglutination is due to a reaction between substances in the red cells called *agglutinogens* and substances in the plasma known as *agglutinins*.

Agglutinogens and agglutinins can not be seen. When they react with each other, however, they produce a clumping of the red cells which is easily observable. A simple analogy would be electricity. We can not see the positive and negative charges but we can see their effects.

The agglutinogens, like the color of a person's eyes, are inherited characteristics and consequently never change. Their presence or absence in the blood enables everyone to be placed in specific blood groups.

100 millimeters the pressure will not cause any noticeable effect on normal capillaries However in essential thrombocytopenic purpura the pressure will cause the capillaries to rupture The escaping blood then produces tiny spots known as petechiæ (Fig 57)

**Procedure for the Capillary Resistance Test**

RUMPEL LEEDE METHOD

1 Using a marking pencil or pen mark any petechiæ already present on the patient's forearm
2 Place the cuff of a blood pressure apparatus above the bend in the elbow Inflate at about 100 millimeters pressure and maintain for 5 minutes
3 Release the pressure and count the number of fresh petechiæ formed (Fig 57)

Agglutinin b is frequently referred to as beta or anti B and agglutinin a is often called alpha or anti A. It is well to keep these synonyms in mind.

### *Table 16.—Agglutinogens and Agglutinins of the Four Major Blood Groups*

| *Blood Group* | *Agglutinogens in Red Cells* | *Agglutinins in Plasma* |
|---|---|---|
| A | A | b |
| B | B | a |
| AB | AB | none |
| O | O | a and b |

When the cells of a group A person are placed in a test tube and plasma or serum from a group B person is added, the red cells become clumped or agglutinated. The agglutination is explained by the presence of agglutinogen A in the red cells of the group A person and the presence of agglutinin a (anti A) in the plasma of the group B individual. Whenever like agglutinogens and agglutinins (A and a, A and ab, B and b, or B and ab) are mixed in a test tube, agglutination takes place. This is followed by hemolysis of the red cells and consequently is of considerable concern in the transfusion of blood.

Although the above agglutination reaction takes place in a test tube, the situation is slightly different in actual practice. In the transfusion of blood, it is found that any agglutinins in the donor's plasma are so diluted by the patient's blood that their ability to clump the cells of the patient is negligible. In fact, certain substances (blood group specific substances A and B) can be added to the donor's blood which will completely neutralize the agglutinins.

The real danger in the transfusion of blood lies in the clumping of the donor's cells by the agglutinins in the patient's plasma. If the agglutinogens in the donor's red cells meet similar agglutinins (A meets a or ab, or B meets b or ab) upon entering the patient, a reaction takes place between the incoming agglutinogens and the agglutinins of the patient. This reaction causes the agglutination and hemolysis

A discussion of the agglutinogens and the blood groups which they give rise to is found on the following pages The material will be arranged in the order indicated below

- *a* The four major blood groups
- *b* Agglutinogens M N and MN
- *c* The Rh factor
- *d* The subgroups

**The Four *Major Blood Groups***

Research workers have named the four major blood groups A B AB and O Every person may be classified in one of these blood groups his specific group being determined by the type of agglutinogens in his red cells An individual in group A has agglutinogen A in his red cells and a person in group B has agglutinogen B People in group AB have both agglutinogen A and agglutinogen B in their red cells and those in group O have neither agglutinogen A nor agglutinogen B The breakdown of the blood groups according to population is given in Table 15

***Table 15 —Breakdown of the Blood Groups***

| *Blood Group* | *Approximate Per Cent of Population* |
|---|---|
| AB | 5 |
| B | 10 |
| A | 40 |
| O | 45 |

When the blood from a group A person is separated into cells and plasma the red cells contain agglutinogen A and the plasma contains a substance which has been given the name agglutinin b Note that the agglutinogen is designated with a capital letter whereas the agglutinin is given a small letter People in the other blood groups have the following agglutinins Those in group B have agglutinin a in their plasma people in group AB have no agglutinins in their plasma and those in group O have both agglutinin b and agglutinin a The above facts are summarized in Table 16 and should be thoroughly understood before proceeding

are of legal interest in paternity tests because every person has one of these agglutinogens and the particular type which he possesses must be inherited from one of his parents For example if a child has agglutinogen M in his red cells agglutinogen M must also be present in the blood of his father or mother

**The Rh Factor**

In 1937 Landsteiner and Wiener were endeavoring to discover new agglutinogens in the red cells of human blood They believed that other agglutinogens were present in addition to agglutinogens A B AB M N and MN Their research confirmed their assumptions and they discovered another agglutinogen Since these men were using rhesus monkeys as experimental animals they called the new agglutinogen the Rh factor—designating it by the first two letters of the word rhesus Further investigation revealed that the Rh factor was present in 85% of the population People having this agglutinogen in their red cells were called Rh positive whereas those lacking it were classified as Rh negative

At this point recall that blood may be separated into cells and plasma and also that blood containing agglutinogen A or agglutinogen B has agglutinins in its plasma The agglutinogen Rh (Rh factor or Rh agglutinogen) differs from agglutinogen A and agglutinogen B in that there are no *naturally occurring* agglutinins in its plasma The Rh agglutinins may be *produced* however by an Rh negative person receiving Rh positive blood The production of the agglutinins is the body s reaction against the introduction of the foreign agglutinogens When the Rh agglutinins are formed they can react with the Rh agglutinogens and cause clumping and hemolysis of the red cells Such conditions may be met in repeated transfusions and pregnancy A discussion of each situation follows

When a patient with Rh negative blood is given a transfusion with Rh positive blood Rh agglutinins are produced in the patient s plasma Repeated transfusions of this nature increase the strength or titer of the Rh agglutinins If

of the donor's cells. The result may be fatal. *Therefore in the transfusion of blood the red cell agglutinogens entering the patient must not meet similar agglutinins.*

Since group O blood has no agglutinogens in its red cells, there is no danger of its meeting similar agglutinins in the patient. It is therefore called the universal donor. On the other hand, because group AB blood contains no agglutinins in its plasma, it can not agglutinate a donor's incoming cells. Consequently, it is called the universal recipient.

It is extremely important that the student grasp the above concepts. A review of the discussion may be obtained by a careful study of Table 17.

***Table 17.—Compatible Donors for the Four Major Blood Groups***

| | Donor's Blood Group | Agglutinogens in Donor's Red Cells | | Agglutinins in Patient's Plasma | Patient's Blood Group | |
|---|---|---|---|---|---|---|
| | A | A | ⟶ | b | A | |
| | B | B | ⟶ | a | B | |
| Universal Donor | O | None | ⟶ | a | B | |
| Universal Donor | O | None | ⟶ | b | A | |
| Universal Donor | O | None | ⟶ | ab | O | |
| Universal Donor | O | None | ⟶ | None | AB | Universal Recipient |
| | A | A | ⟶ | None | AB | Universal Recipient |
| | B | B | ⟶ | None | AB | Universal Recipient |
| | AB | AB | ⟶ | None | AB | Universal Recipient |

## Agglutinogens M, N, and MN

The presence of agglutinogen M, agglutinogen N, and agglutinogen MN (both M and N) have recently been discovered in human blood. Every individual, in addition to belonging to one of the four major blood groups, possesses agglutinogen M, N, or MN. These agglutinogens differ from agglutinogen A and agglutinogen B in that they have no corresponding agglutinins, m and n, in their plasma. Since the presence of agglutinins is necessary for agglutination to take place, these newly discovered agglutinogens are never responsible for the clumping of red cells. Consequently, they are of no concern in blood transfusions. However, they

in the blood alone determines whether a person is Rh positive or Rh negative. Consequently in routine laboratory work tests are made only for the $Rh_0$ factor. Since the various subdivisions of the Rh factor are not of clinical significance they will not be discussed in detail. For further information the interested student is referred to the current literature in the field and the present day research papers of Wiener, Fisher, Race and others.

### The Subgroups

Research workers have recently found that agglutinogen A exists in two forms $A_1$ and $A_2$. This means that group A blood is divided into subgroup $A_1$ and subgroup $A_2$. Further investigation revealed the fact that 80% of the people typed as group A really belong to subgroup $A_1$ and the remaining 20% belong to subgroup $A_2$. This discovery also divides group AB blood into subgroup $A_1B$ and subgroup $A_2B$.

It so happens that agglutinogens $A_1$ and $A_2$ are so closely related that they are of little concern in the transfusion of blood. Thus group $A_1$ blood may be given to a group $A_2$ patient and group $A_1B$ blood may be given to a group $A_2B$ individual. However their significance and chief danger lies in the fact that agglutinogen $A_2$ has a weak agglutinating power. Consequently when group $A_2$ blood is typed the agglutinogens in the red cells may be so weak that the cells fail to agglutinate. Such blood would then erroneously be typed as group O. Also if agglutinogen $A_2$ were too weak to react, $A_2B$ blood would be mistakenly typed as group B. These typing errors however are usually eliminated by using group A typing serum of a very high titer which contains both agglutinogen $A_1$ and agglutinogen $A_2$. The cross match prior to a transfusion also serves to detect any such typing errors.

Since the subgroups of group A and group AB blood are of little concern in blood transfusions they are not determined routinely by the clinical laboratory. If it is desirable to find

A well written booklet discussing the present status of the Rh factor may be obtained free of charge from the Ortho Pharmaceutical Corp, Raritan, N J.

the titer becomes sufficiently high further transfusion with Rh positive blood will bring about a reaction between the patient s newly formed Rh agglutinins and the donor s incoming Rh agglutinogens The reaction causes the agglutination and hemolysis of the red cells entering the patient The result may be fatal Therefore before a transfusion the patient s blood is tested for the presence of the Rh factor The Rh positive patients are given Rh positive blood and the Rh negative individuals are given Rh negative blood This eliminates transfusion reactions which were formerly due to the Rh factor and writes another brilliant page in medical research

When an Rh negative woman is carrying an Rh positive fetus* the fetus s blood may mingle (possibly through a tear in the placenta) with the mother s blood The Rh agglutinogens in the fetus s red cells cause the production of Rh agglutinins in the mother s plasma These newly formed agglutinins are then carried into the fetus via the circulatory system A steady stream of Rh agglutinins from the mother pours into the fetus This increases the strength or titer of these foreign Rh agglutinins in the fetus s plasma Finally a reaction takes place within the fetus between the Rh agglutinogens in its red cells and the Rh agglutinins which came from the mother This reaction results in the agglutination and hemolysis of the fetus s red cells The infant is then born with a severe and often fatal anemia (erythroblastosis fetalis) The treatment in mild cases is to give immediate transfusions with Rh negative blood and in severe cases to drain the infant s entire blood supply and simultaneously replace it with Rh negative blood

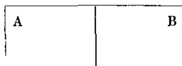
In recent years several varieties of the Rh factor have been discovered These are classified as $Rh_1$ $Rh_2$ etc with the original Rh factor receiving the label $Rh_0$ The $Rh_0$ agglutinogen has a much stronger agglutinating power than the other Rh agglutinogens Because of this greater antigenicity it is the most important for its presence or absence

* In the vast majority of cases where the following reaction occurs the mother is Rh negative and the fetus is Rh positive However cases have been recorded where the reverse is true the mother being Rh positive and the fetus being Rh negative

**Slide Method for ABO Grouping**

A saline cell suspension may be used in place of the oxalated blood mentioned below If the saline cell suspension is used cover the preparation with a Petri dish and allow 30 minutes to elapse before making the reading

1 Get a glass slide and draw a line through the center with a wax pencil Label one square A and the other B Thus




2 Place 2 drops of oxalated blood in each square
3 To the blood in square A add 1 drop of group A (anti B) typing serum Mix with an applicator stick
4 To the blood in square B add 1 drop of group B (anti A) typing serum Mix with an applicator stick
5 Examine for clumping of the cells If there is clumping in square B and none in square A the blood belongs to group A If there is clumping in square A and none in square B the blood belongs to group B Clumping in both squares places the blood in group AB whereas no clumping in either square indicates group O These results are illustrated in Figure 58

**Test Tube Method for ABO Grouping**

1 Get two small test tubes Write the patient s name on the tubes and label one tube A and the other B Place 2 drops of the patient s cell suspension in each tube

the subgroups a serum called Absorbed B serum is used Certain agglutinins have been absorbed from this serum so that it will clump cells containing agglutinogen $A_1$ but will not clump cells containing agglutinogen $A_2$ Thus the cells of group $A_1$ and group $A_1B$ will be agglutinated by Absorbed B serum whereas the cells of group A and group $A_2B$ will not be agglutinated

## ✓ ABO GROUPING

The 4 major blood groups are commonly referred to as the ABO group And the procedure for finding a person s specific group is known as ABO grouping This section discusses (1) the preparation of blood for ABO grouping (2) the slide method for ABO grouping and (3) the test tube method for ABO grouping

### Preparation of Blood for ABO Grouping

Either oxalated blood or a cell suspension may be used for ABO grouping If oxalated blood is employed the results may be read immediately If a cell suspension is used the cells are less concentrated and the reaction may require about 30 minutes

*If oxalated blood is to be used get an oxalated tube and* materials for a venipuncture Write the patient s name on the oxalated tube Make a venipuncture withdraw about 5 cc of blood and transfer to the tube Place the cork in the mouth of the tube and invert about 8 to 10 times to dissolve the oxalate

If a saline cell suspension is to be used get a Kahn tube and materials for a finger puncture Write the patient s name on the Kahn tube and place about 6 cc of normal saline in the tube Make a finger puncture wipe away the first drop of blood and add a few drops to the tube Place a cork in the mouth of the tube and invert several times in order to mix The cell suspension should not be too dilute or too concentrated When a test tube containing a properly made cell suspension is held directly over printed material the print should be *barely discernible*

**Slide Method for ABO Grouping**

A saline cell suspension may be used in place of the oxalated blood mentioned below If the saline cell suspension is used cover the preparation with a Petri dish and allow 30 minutes to elapse before making the reading

1 Get a glass slide and draw a line through the center with a wax pencil Label one square A and the other B Thus

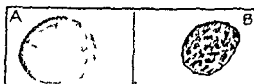
| | |
|---|---|
| A | B |

2 Place 2 drops of oxalated blood in each square
3 To the blood in square A add 1 drop of group A (anti B) typing serum Mix with an applicator stick
4 To the blood in square B add 1 drop of group B (anti A) typing serum Mix with an applicator stick
5 Examine for clumping of the cells If there is clumping in square B and none in square A the blood belongs to group A If there is clumping in square A and none in square B the blood belongs to group B Clumping in both squares places the blood in group AB whereas no clumping in either square indicates group O These results are illustrated in Figure 58

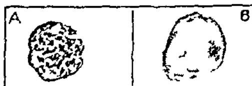
**Test Tube Method for ABO Grouping**

1 Get two small test tubes Write the patient s name on the tubes and label one tube A and the other B Place 2 drops of the patient s cell suspension in each tube

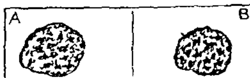
Group A Serum (Anti B) | Group B Serum (Anti A)



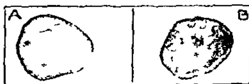

Group A blood




Group B blood




Group AB blood




Group O blood

*Fig. [illegible]8 —Grouping the patient's blood*

2 To the cell suspension in tube A add 1 drop of group A (anti B) typing serum Shake gently to mix
3 To the cell suspension in tube B add 1 drop of group B (anti A) typing serum Shake gently to mix
4 Let the tubes stand at room temperature for 15 minutes
5 Centrifuge at *low* speed for 1 minute Re suspend the cells by gently shaking the tubes
6 Examine for clumping If in doubt place on a glass slide and examine with the low power (16 mm ) objective of the microscope If there is clumping in tube B and none in tube A the blood belongs to group A If there is clumping in tube A and none in tube B the blood belongs to group B Clumping in both tubes places the blood in group AB whereas no clumping in either tube indicates group O blood These results are illustrated in Figure 58

## Rh TYPING

The slide method and the test tube method are used to determine the Rh factor The slide method is much faster requiring only a few minutes whereas the test tube method takes about one half hour The slide method however is not as sensitive and may fail to pick up weak reactions Any negative results with this procedure are therefore checked with the more sensitive test tube method The procedures follow

**Slide Method of Rh Typing**

1 Warm a slide on the microscope lamp until it reaches approximately body temperature (37 C ) (Many laboratories have a view box which maintains the proper temperature )

2 Using either a medicine dropper or 2 applicator sticks held together between the thumb and forefinger place a large drop of oxalated blood on the left side of the slide (Fig 59) If desirable blood obtained from a finger puncture may be used in place of the oxalated blood

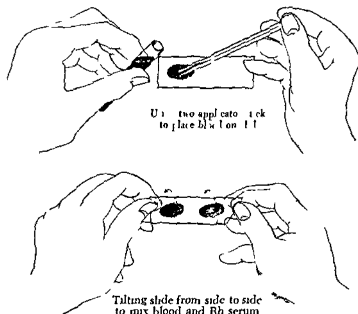



Fig 59 —Testing for the Rh factor

3 Spread the drop of blood so that it covers an oval area about the size of a nickel
4 Place another large drop of blood on the right side of the slide and spread in a similar manner This latter drop will be used as a control to compare with a positive test
5 Add a drop of anti $Rh_0$ serum (labelled for slide method) to the drop of blood on the

left side Add nothing to the drop on the right side

6 Tilt the slide from side to side to mix the blood and serum and at the same time look for a fine granular clumping of the cells (Fig 59) If clumping is going to take place it occurs within 1 to 4 minutes Do not confuse true granular clumping with a false coarse clumping which occurs when blood begins to dry up (Fig 60)



*Rh positive*



*Rh negative*



*False positive (drying blood)*

Fig 60 —Positive and negative tests for the Rh factor (slide method)

7 If clumping occurs record the test as Rh positive If no clumping takes place record the test as Rh negative Should the test be negative perform the more sensitive test tube method given below

**Test Tube Method of Rh Typing**

1 Make a serum cell suspension as follows To about 3 cc of the patient s serum add 1 drop of the patient s blood Mix (The above cell suspension may also be obtained by *gently* shaking a test tube containing the patient s blood clot and serum The suspension however should not be too concentrated a 2% suspension being most suitable)

2 Place 2 drop of the above serum cell suspension in a small test tube

3. Add 2 drops of anti Rh$_0$ typing serum (labelled for test tube method) Shake gently and place in a 37° C water bath for 30 minutes
4. Centrifuge at *low* speed for 1 minute
5. Resuspend the cells by gentle shaking transfer to a glass slide, and examine under the low power (16 mm ) objective of the microscope
6. If clumping is present record the test as Rh positive If clumping is absent record the test as Rh negative

## ✓CROSS MATCH

When an order comes into the laboratory to type and cross match blood for a transfusion the technician is entrusted with a task which may save a life Yet a single mistake in the procedure may cause incompatible blood to be given and the resulting hemolysis of cells might easily cost a life Consequently it is the duty of the technician to work with extreme diligence for he often bridges the gap between life and death

A cross match determines the compatibility of the donor s blood and the patient s blood It consists of two parts The first part is called the major side and the second part is called the minor side

The major side is used to test the compatibility of the donor s cells with the patient s serum This side is the most important for it contains the donor s red cell agglutinogens These agglutinogens the student will recall must not meet similar agglutinins in the patient If they do the red cells clump or agglutinate Any clumping of cells on the major side completely rules out the donor s blood

The minor side is used to test the compatibility of the donor s serum with the patient s cells This side contains the donor s serum agglutinins Upon entering the patient these agglutinins will be greatly diluted by the patient s blood Therefore their effect if any on the patient s red cell agglutinogens will be negligible Theoretically a slight clumping

on the minor side is permissible but in actual practice any such agglutination al o rules out the donor s blood

The following pages first discuss the blood preparations required for a cross match and then give the actual procedure Following the instructions the sources of error are listed and discussed These sources of error should be thoroughly understood by the student

## Preparation for a Cross Match

Four blood preparations are needed for a cross match They are listed below and the manner in which they are obtained or prepared follows

*a* Donor s cell suspen ion
*b* Donor s serum
*c* Patient s cell suspension
*d* Patient s serum *

### Donor s Cell Suspension and Serum

When blood is furni hed by a blood bank pilot tubes containing the donor s cell suspension and serum accompany the bottle of blood If the blood is obtained from a donor without the aid of a blood bank the cell suspension and serum are prepared when blood is withdrawn The procedure for their preparation is carried out in the same manner as that given below for the patient

### Preparation of Patient s Cell Suspension

Write the patient s name on a Kahn tube and place about 6 cc of normal saline in the tube Add two drops of the patient s blood (either oxalated or whole blood) to the tube Place a cork in the mouth of the tube and invert several times in order to mix The cell suspension should not be too dilute or too concentrated When the tube containing a

Some technician use the patient s pla ma rath r than serum Both are equally satisfactory If plasma is to be used obtain the patient s plasma in place of serum and substitute the word pla ma for serum in the procedu e

properly made cell suspension is held directly over printed matter the print should be barely discernible

### Preparation of Patient's Serum

Get materials for a venipuncture and a Kahn tube Label the tube with the patient s name Withdraw about 5 cc of blood from the patient transfer to the tube and allow to clot Centrifuge Pour off the serum into a test tube Label the tube with the patient s name

#### Procedure for a Cross Match

1 Get 6 Kahn tubes and write the patient s name on each tube Label 3 tubes D C + P S This stands for donor s cells plus patient s serum It is the major side Label the other 3 tubes P C + D S This stands for patient s cells plus donor s serum and is the minor side Arrange the 6 tubes in a rack as shown in Figure 61 Get the donor s cell suspension and serum and the patient s cell suspension and serum (preparation given above) Also set them in the rack as indicated in Figure 61
2 To set up the major side
Using 1 cc pipets place 0 1 cc of the donor s cell suspension in each of the 3 tubes labeled D C + P S Add 0 1 cc of the patient s serum to each tube Shake the tubes gently in order to mix
3 To set up the minor side
Using 1 cc pipets place 0 1 cc of the patient s cell suspension in each of the 3 tubes labelled P C + D S Add 0 1 cc of the donor s serum to each tube Shake gently in order to mix
4 Place 1 tube from the major side and 1 tube from the minor side in the refrigerator Place 1 tube from the major side and 1 tube from the minor side in a 37 C waterbath

Let the 2 remaining tubes stand at room temperature

5 In 30 minutes (60 minutes for infants since they have a lower titer of agglutinins) centrifuge the tubes at *low* speed for 1 minute Note the color of the fluid above the cells A pink tinge indicates that hemolysis has taken place and consequently the bloods are incompatible If the fluid above the cells is clear the cells must then be inspected

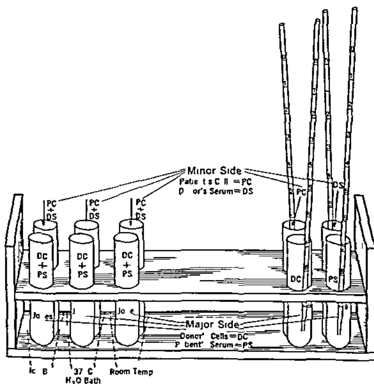



FIG 61 —Set-up for a cross match

for agglutination. *Gently* shake the tubes to resuspend the cells and place the contents of each tube on a separate glass slide. Examine with the high dry (4 mm.) objective of the microscope. Record any clumping







Fig. 62.—Positive, negative and false agglutination. *A*, Positive agglutination. *B*, Negative agglutination. *C*, False agglutination and rouleaux formation. (Kolmer's *Immunity and Biologic Therapy*, courtesy of W. B. Saunders Co.)

(Fig 62) being sure to designate the test tube involved Make the report as follows

6 Compatible bloods No clumping in any of the 6 tubes

Incompatible bloods Clumping in any of the tubes which were placed at 37 C temperature or stood at room temperature

Doubtful reaction If clumping occurs in the refrigerated tubes it indicates the presence of either cold agglutinins or autoagglutinins Both types of agglutinins react best at cold temperatures Agglutinations of this nature are rare but should they occur consult the physician He may reject the blood or simply order it to be given slowly thus giving the blood a chance to warm up before entering the patient

## Sources of Error in Typing and Cross Matching Blood

*It is very important that the student learn and thoroughly* understand the following sources of error in typing and cross matching blood These sources of error may serve the student in several ways First they may be used as handy guideposts in carrying out the procedure Second they may be used to help recognize the significant factors in the transfusion of blood Incidentally they also make good examination questions

1 *Typing and cross matching the wrong blood*

To illustrate Suppose that you are drawing the blood for 2 typings and cross matchings and forget to label the tubes with the patient s name You do the 2 typings order the blood and get compatible cross matches The blood is given and both patients have transfusion reactions (which may be fatal!) because you failed to label the tubes and got the blood mixed up This is perhaps the most serious mistake a technician can make since there is no way to pick up the error *Therefore always label the tubes with the patient s name*

2 *Making the saline cell suspension too weak or too concentrated*

If the cells are too far apart the agglutinating force may not be able to bring them together If the cells are too close rouleaux formation (red cells bunched together like stacks of coins) may take place This is commonly mistaken for agglutination It may be corrected by diluting the cells and serum with a small drop of normal saline

3 *The use of saline cell suspensions which have stood for several days*

When cell suspensions stand for long periods of time the cells lose their ability to become agglutinated Cell suspensions should be made fresh for each typing and cross match

4 *The use of weak or contaminated typing serums*

The commercial typing serums are made from pooled serums of high titer and usually can be relied upon to have a sufficiently high titer Contamination can be prevented by putting nothing into the typing serum except the dropper which comes with the bottle

5 *Interchanging the typing serums*

If the typing serums are interchanged group A blood would be typed as group B and vice versa In order to guard against this some technicians place the typing serum bottles in a Petri dish and pour in liquid paraffin When the paraffin hardens it fixes the bottles in position This lessens the possibility of getting them mixed up

6 *Failure to pipet the donor s and patient s serums and cells into the correct tubes*

This is a serious mistake since it is difficult to detect It may be prevented by paying strict attention to the job and double checking each step

7 *Failure to allow sufficient time for agglutination to take place*

In typing blood The agglutinogen $A_2$ in subgroups $A_2$ and $A_2B$ has a weak agglutinating power and is slow to react

Therefore if the typing is read too soon group $A_2$ blood may be mistaken for group O and group $A_2B$ blood may be mistaken for group B

In cross matching blood Infants usually have much weaker agglutinins than adults Weak agglutinins require a longer period of time to react If sufficient time is not allowed a weak reaction may not be detected and incompatible blood may be given

8 *Failure to give the blood that was cross matched*

In most hospitals a nurse calls for the blood and takes it to the ward to give to the patient All bottles of blood may look the same to her Make certain that she gets the blood that you cross matched

9 *Failure to perform the proper cross matches during multiple transfusions*

When a series of transfusions are to be given a patient two factors should be observed First all bottles of blood which are to be given should be cross matched with each other second each bottle should be cross matched with the patient just before it is given

## COOMBS TEST

There are two Coombs tests the Direct Coombs and the Indirect Coombs

The Direct Coombs test is usually used to determine the presence or absence of blocking antibodies These antibodies may coat the red cells of an infant suspected of having erythroblastosis fetalis If the antibodies are present it increases the physician s suspicion that the infant may be an erythroblastotic baby In such cases he may order an immediate exchange transfusion This will remove the infant s blood before the hemolyzing red cells have an opportunity to do any serious damage

The Indirect Coombs test is also used to establish the presence or absence of antibodies This test however examines the serum of a mother who gives birth to an infant

suspected of having erythroblastosis fetalis If the antibodies are present the test is positive This increases the possibility of an erythroblastotic baby

The procedures for the Direct Coombs and the Indirect Coombs are given below

### Direct Coombs Test

1 Get a Kahn tube and add about 6 cc of *normal (0 85%) saline Get materials for a* finger puncture
2 Puncture the infant s big toe and add about 2 drops of blood to the saline tube (Cord blood may also be used ) Invert the tube *several times to mix*
3 Wash the above cells 3 times in the following manner Centrifuge the cell suspension Pour off the fluid Add about 6 cc of normal saline Mix
4 *After discarding the fluid from the third* washing remove as much saline as possible by allowing the tube to drain over a gauze pad for a few seconds
5 By adding normal saline adjust the concen*tration of cells so that you have about a 2%* suspension When a test tube containing a *2%* suspension of *cells* is held directly over printed matter the print is barely discernible
6 Place 2 drops of the above 2% cell suspension in a small test tube
7 Add 2 drops of the Coombs serum Mix
8 Let stand at room temperature for 15 minutes
9 Centrifuge at *low* speed for 1 minute
10 Resuspend *the cells by gently shaking the* tube and examine for clumping of cells If *you are in doubt regarding the clumping* place the cells on a glass slide and examine with the low power (16 mm ) objective of the microscope

11 If the cells are clumped report the test as positive If the cells are not clumped report the test as negative
12 NOTE Some laboratories run the following controls
(a) a negative control with group O Rh negative cells
(b) a positive control with group O Rh positive cells These cells are first sensitized by adding 2 drops of diluted (1 to 3) anti $Rh_0$ slide test serum and incubating at 37 C for one-half hour

### Indirect Coombs Test

1 Get materials for a venipuncture and a Kahn tube
2 Make a venipuncture on the patient withdraw about 6 cc of blood and transfer to the Kahn tube
3 Allow the blood to clot and centrifuge to obtain the serum
4 Prepare a 2% normal saline suspension of selected test cells (washed group O Rh positive cells)
5 Place 2 drops of the above 2% suspension in a small test tube
6 Add 2 drops of the patient s serum Mix
7 Place in a 37 C water bath for 60 minutes
8 Remove the tube from the water bath and wash the cells 3 times as follows Add about 6 cc of normal saline mix centrifuge and pour off the fluid
9 After the third washing allow the tube to drain in order to remove as much saline as possible
10 Add 2 drops of Coombs serum to the cells in the bottom of the tube Mix well
11 Centrifuge at *low* speed for 1 minute
12 Resuspend the cells by gently shaking the tube

suspected of having erythroblastosis fetalis If the antibodies are present the test is positive This increases the possibility of an erythroblastotic baby

The procedures for the Direct Coombs and the Indirect Coombs are given below

### Direct Coombs Test

1 Get a Kahn tube and add about 6 cc of normal (0 85%) saline Get materials for a finger puncture
2 Puncture the infant s big toe and add about 2 drops of blood to the saline tube (Cord blood may also be used) Invert the tube several times to mix
3 Wash the above cells 3 times in the following manner Centrifuge the cell suspension Pour off the fluid Add about 6 cc of normal saline Mix
4 After discarding the fluid from the third washing remove as much saline as possible by allowing the tube to drain over a gauze pad for a few seconds
5 By adding normal saline adjust the concentration of cells so that you have about a 2% suspension When a test tube containing a 2% suspension of cells is held directly over printed matter the print is barely discernible
6 Place 2 drops of the above 2% cell suspension in a small test tube
7 Add 2 drops of the Coombs serum Mix
8 Let stand at room temperature for 15 minutes
9 Centrifuge at *low* speed for 1 minute
10 Resuspend the cells by gently shaking the tube and examine for clumping of cells If you are in doubt regarding the clumping place the cells on a glass slide and examine with the low power (16 mm) objective of the microscope

11 If the cells are clumped report the test as positive If the cells are not clumped report the test as negative
12 NOTE Some laboratories run the following controls
(a) a negative control with group O Rh negative cells
(b) a positive control with group O Rh positive cells These cells are first sensitized by adding 2 drops of diluted (1 to 3) anti $Rh_0$ slide test serum and incubating at 37 C for one half hour

**Indirect Coombs Test**

1 Get materials for a venipuncture and a Kahn tube
2 Make a venipuncture on the patient with draw about 6 cc of blood and transfer to the Kahn tube
3 Allow the blood to clot and centrifuge to obtain the serum
4 Prepare a 2% normal saline suspension of selected test cells (washed group O Rh positive cells)
5 Place 2 drops of the above 2% suspension in a small test tube
6 Add 2 drops of the patient s serum Mix
7 Place in a 37 C water bath for 60 minutes
8 Remove the tube from the water bath and wash the cells 3 times as follows Add about 6 cc of normal saline mix centrifuge and pour off the fluid
9 After the third washing allow the tube to drain in order to remove as much saline as possible
10 Add 2 drops of Coombs serum to the cells in the bottom of the tube Mix well
11 Centrifuge at *low* speed for 1 minute
12 Resuspend the cells by gently shaking the tube

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1 Get a Kahn tube and add about 6 cc of normal (0 85%) saline Get materials for a finger puncture
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3 Wash the above cells 3 times in the following manner Centrifuge the cell suspension Pour off the fluid Add about 6 cc of normal saline Mix
4 After discarding the fluid from the third washing remove as much saline as possible by allowing the tube to drain over a gauze pad for a few seconds
5 By adding normal saline adjust the concentration of cells so that you have about a 2% suspension When a test tube containing a 2% suspension of cells is held directly over printed matter the print is barely discernible
6 Place 2 drops of the above 2% cell suspension in a small test tube
7 Add 2 drops of the Coombs serum Mix
8 Let stand at room temperature for 15 minutes
9 Centrifuge at *low* speed for 1 minute
10 Resuspend the cells by gently shaking the tube and examine for clumping of cells If you are in doubt regarding the clumping place the cells on a glass slide and examine with the low power (16 mm ) objective of the microscope

5 Place 0 1 cc of normal (0 85%) salıne ın tubes 2 through 10
6 Place 0 1 cc of the patıent s serum ın tubes 1 and 2
7 Mix the serum and salıne ın tube 2 and trans fer 0 1 cc to tube 3
8 Mix the serum and salıne ın tube 3 and transfer 0 1 cc to tube 4
9 Mix the serum and salıne ın tube 4 and transfer 0 1 cc to tube 5 Contınue this process of dilution through tube 10 After you have mixed tube 10 remove 0 1 cc from the tube and discard
10 Get the fresh 2% salıne cell suspensıon of male group O Rh positive blood
11 Add 2 drops of the above 2% cell suspension to each of the 10 tubes Mix gently (Save the cell suspension as ıt ıs used ın step 18 )
12 *Place the rack ın a 37 C ıncubator or water* bath for 1 hour or longer
13 Get 10 more Kahn tubes Number them 1 to 10 and place ın another test tube rack Label this rack *For Albumin Agglutinins*
14 Place 0 1 cc of pooled male serum ın tubes 2 through 10
15 Place 0 1 cc of the patıent s serum ın tubes 1 and 2
16 Mix the contents of tube 2 and transfer 0 1 cc to tube 3
17 Mix the contents of tube 3 and transfer 0 1 cc to tube 4 Continue this dilution process through tube 10 After you have mixed tube 10 remove 0 1 cc and discard
18 Get the fresh 2% salıne suspension of male group O Rh positive cells Centrifuge Discard the fluid and allow the tube to draın To the cells ın the bottom of the tube add 4 cc of bovine albumin Mix well
19 Add 2 drops of the above albumın suspended cells to each of the 10 tubes Mix gently

13 Examine for clumping If you are in doubt regarding the clumping place the cells on a glass slide and examine with the low power (16 mm ) objective of the microscope
14 If clumping is present report the test as positive If clumping is absent report the test as negative
15 NOTE Some laboratories run positive and negative controls for the above test For detailed instructions consult the literature accompanying your bottle of Coombs serum

## RH TITER

If a pregnant woman is Rh negative and her husband is Rh positive there is a possibility that she may bear an erythroblastotic baby In some cases the strength of Rh antibodies in her serum increases this possibility The strength of Rh antibodies may be determined by an Rh titer The test is performed as indicated below

The following solutions and reagents are needed for this test normal (0 85%) saline fresh male group O Rh positive blood pooled male serum and bovine albumin The male blood and serum may be obtained from students or patients The bovine albumin may be obtained from your local medical supply house

1 Get materials for a venipuncture and a Kahn tube
2 Make a venipuncture on the patient with draw about 6 cc of blood and transfer to the Kahn tube
3 Allow the blood to clot and centrifuge to obtain the serum Obtain fresh male group O Rh positive blood and make a 2% saline cell suspension
4 Get 10 Kahn tubes Number the tubes 1 to 10 and place in a test tube rack Label the rack *For Saline Agglutinins*

5 Place 0 1 cc of normal (0 85%) saline in tubes 2 through 10
6 Place 0 1 cc of the patient s serum in tubes 1 and 2
7 Mix the serum and saline in tube 2 and transfer 0 1 cc to tube 3
8 Mix the serum and saline in tube 3 and transfer 0 1 cc to tube 4
9 Mix the serum and saline in tube 4 and transfer 0 1 cc to tube 5 Continue this process of dilution through tube 10 After you have mixed tube 10 remove 0 1 cc from the tube and discard
10 Get the fresh 2% saline cell suspension of male group O Rh positive blood
11 Add 2 drops of the above 2% cell suspension to each of the 10 tubes Mix gently (Save the cell suspension as it is used in step 18 )
12 Place the rack in a 37 C incubator or water bath for 1 hour or longer
13 Get 10 more Kahn tubes Number them 1 to 10 and place in another test tube rack Label this rack *For Albumin Agglutinins*
14 Place 0 1 cc of pooled male serum in tubes 2 through 10
15 Place 0 1 cc of the patient s serum in tubes 1 and 2
16 Mix the contents of tube 2 and transfer 0 1 cc to tube 3
17 Mix the contents of tube 3 and transfer 0 1 cc to tube 4 Continue this dilution process through tube 10 After you have mixed tube 10 remove 0 1 cc and discard
18 Get the fresh 2% saline suspension of male group O Rh positive cells Centrifuge Discard the fluid and allow the tube to drain To the cells in the bottom of the tube add 4 cc of bovine albumin Mix well
19 Add 2 drops of the above albumin suspended cells to each of the 10 tubes Mix gently

20. Place in a 37° C incubator or water bath for 1 hour.
21. After the hour has elapsed, remove the rack labelled *For Saline Agglutinins* from the incubator. Also remove the rack labelled *For Albumin Agglutinins* from the incubator. Shake the racks gently in order to mix the contents of the tubes.
22. Begin with tube 1 of each set of tubes and examine for clumping in the following manner. Place the contents of each tube on a glass slide and observe under the low power (16 mm.) objective of the microscope.
23. Record the tubes which show clumping.
24. Report the tube numbers and the highest dilution which show clumping (see Table 18).

***Table 18.—Dilutions of Tubes in Rh Titer***

| *Tube Number* | *Dilution* |
|---|---|
| 1 | 1:1 |
| 2 | 1:2 |
| 3 | 1:4 |
| 4 | 1:8 |
| 5 | 1:16 |
| 6 | 1:32 |
| 7 | 1:64 |
| 8 | 1:128 |
| 9 | 1:256 |
| 10 | 1:512 |

25. A sample report is given below.

REPORT OF RH TITER

| | |
|---|---|
| For Saline Agglutinins | Clumping in tubes 1 through 5 |
| | Highest dilution is 1:16 |
| For Albumin Agglutinins | No clumping found |

Chapter 6

# Miscellaneous Tests

This chapter presents those tests which do not fit into the previous categories. The examinations discussed are listed below.

- L E Cells
- Eosinophil Count
- Spinal Cell Count
- Malaria Parasites
- Bone Marrow Smears
- Heterophile Antibody Test

## L E CELLS

### Information Significant to the Student

Lupus erythematosus is a disease of the skin which is characterized by eruptions on the surface of the body. Its cause is unknown. The disease is sometimes difficult to diagnose. A recent laboratory test however has come to the aid of the physician. This test consists of examining the blood or bone marrow for L E cells. The procedure is given below.

The L E cell derives its name from the disease—lupus erythematosus. The cell consists of a mass of nuclear material surrounded by one or more segmented cells (Fig 63.)

It is believed by some that the formation of the cell takes place in two stages. First there is an abnormal substance in the plasma. This substance breaks down the cytoplasm of lymphocytes and leaves a distorted nucleus. Second this distorted nucleus is engulfed or phagocytized by segmented cells.

20 Place in a 37° C incubator or water bath for 1 hour
21 After the hour has elapsed remove the rack labelled *For Saline Agglutinins* from the incubator Also remove the rack labelled *For Albumin Agglutinins* from the incubator Shake the racks gently in order to mix the contents of the tubes
22 Begin with tube 1 of each set of tubes and examine for clumping in the following manner Place the contents of each tube on a glass slide and observe under the low power (16 mm ) objective of the microscope
23 Record the tubes which show clumping
24 Report the tube numbers and the highest dilution which show clumping (see Table 18)

***Table 18 —Dilutions of Tubes in Rh Titer***

| *Tube Number* | *Dilution* |
|---|---|
| 1 | 1 1 |
| 2 | 1 2 |
| 3 | 1 4 |
| 4 | 1 8 |
| 5 | 1 16 |
| 6 | 1 32 |
| 7 | 1 64 |
| 8 | 1 128 |
| 9 | 1 256 |
| 10 | 1 512 |

25 A sample report is given below

REPORT OF RH TITER

| | |
|---|---|
| For Saline Agglutinins | Clumping in tubes 1 through 5 |
| | Highest dilution is 1 16 |
| For Albumin Agglutinins | No clumping found |

4 Pour off and discard the serum
5 Using 2 applicator sticks *thoroughly* mash up the blood clot (As an alternate procedure the clot may be passed through a #40 wire mesh)
6 Discard the clot and centrifuge the bloody serum
7 Transfer 1 cc of the top layer of cells to a Wintrobe hematocrit tube
8 Centrifuge for 10 minutes
9 Using a fine capillary pipet remove the serum which is above the thin white layer of cells *Discard this serum*
10 Using the capillary pipet carefully transfer the thin white layer of cells to a clean glass slide
11 Make blood smears in the usual manner Allow the smears to dry
12 Stain with Wright s stain
13 *Examine with the oil immersion objective of* the microscope The L E cell is illustrated in Fig 63
14 Report the presence or absence of L E cells making sure to examine the slide thoroughly

## EOSINOPHIL COUNT

### Information Significant to the Student

The eosinophil count is used in diagnosing diseases of the adrenal gland For example when a healthy person is given an injection of the hormone ACTH the eosinophil count drops below the normal values of 150 to 300 cells per cubic millimeter In diseases of the adrenal gland however the count remains the same

### Procedure for the Eosinophil Count

The eosinophil count is made in a manner somewhat similar to a white cell count In order to increase the accuracy

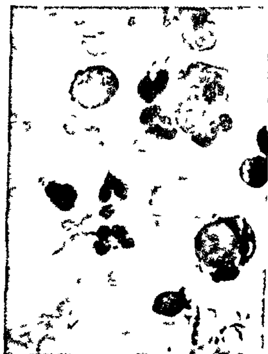


Fig 63 —Lupus Erythematosus (L E ) Cells The cluster in the upper right hand corner is a typical rosette with an L E body in the center The cell containing a body in the lower right hand corner is a tart cell which is not pathognomonic of lupus erythematosus Source of material bone marrow × 800 From Gradwohl R B H *Clinical Laboratory Methods and Diagnosis* 5th Edition courtesy of the C V Mosby Company )

**Procedure for Detecting L E Cells**

1 Get materials for a venipuncture and a Kahn tube
2 Make a venipuncture on the patient with draw about 8 cc of blood and transfer to the Kahn tube
3 Allow the blood to stand at room temperature for 2 hours (If a 37 C incubator is available place blood in incubator )

of 1 0/0 9 Multiplying the dilution correction factor of 10 by the volume correction factor of 1 0 0 9 gives us the figure 11 )

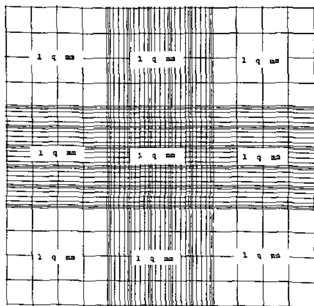



FIG 64 —Total ruled area to b counted in the eo nophil count and sp nal cell count The total ruled ar a is 9 sq mm Th counting chamber is 0 1 mm de p Therefore tl total volume is 9 × 0 1 = 0 9 u mm (Illu tration cou te y of Bau ch & Lomb Co )

11 Example of calculation

One side of the counting chamber had a count of 10 The other side had a count of 8 Therefore

10 + 8 = 18 total count

And
18 divided by 2 = 9 average count

however some technicians fill 2 pipets make 2 separate counts and average the two

There are several diluting fluids which can be used for the eosinophil count In the following procedure Tannen s diluting fluid is used In place of this however any other eosinophil diluting fluid may be used

1 Get materials for a finger puncture a white cell pipet, and a bottle of Tannen s diluting fluid (preparation given in the Appendix on page 242)
2 Make a finger puncture wipe away the first drop and draw blood to the 1 0 mark Now draw diluting fluid to the 11 0 mark
3 Let stand 15 minutes
4 Mix the contents of the pipet as you would for a white cell count
5 Discard the first 4 drops and load *both* sides of the counting chamber
6 Using the low power (16 mm ) objective of the microscope scan several fields and note that the eosinophils are red in color
7 Count the eosinophils in the *entire* ruled area on one side of the counting chamber This will be the nine 1 sq mm sections illustrated in Figure 64 Record the count
8 Now count the eosinophils in the *entire* ruled area on the other side of the counting chamber Record the count
9 Add the counts from each side of the counting chamber to get the total count Now divide the total count by 2 to get the average count
10 Multiply the average count by 11
(The figure 11 represents 2 correction factors First the blood was diluted 1 in 10 and thus we have the dilution correction factor of 10 Second the cells were counted in 0 9 cubic millimeter and the report is to be given as the number in 1 0 cubic millimeter Thus we have a volume correction factor

3 Using the low power (16 mm) objective *count the cells in the entire ruled area* This will be the nine 1 sq mm sections illustrated in Figure 64 page 183

4 Calculations
By counting the cells in the entire ruled area you have counted *the number in 0 9 cu mm* The report however is to be given as the number in 1 0 cu mm Therefore multiply the number counted by 1 0/0 9 (or 10/9)

5 Example
The number of cells counted in the entire ruled area was 9 Multiplying this by 10/9 we have

$9 \times 10/9 = 10$ cells per cu mm

6 After the count is made the counting chamber *should be soaked in alcohol to prevent contagion*

7 Note After some experience a differential cell count may be made by examining the cells on the counting chamber

**Differential Cell Count**

If the total cell count is within the normal range of 0 to 10 there is no point in making a differential cell count If however the count is elevated a differential cell count should be made

The white cells usually found in spinal fluid are lymphocytes and segmented cells

The lymphocytes usually outnumber the segmented cells in the following diseases tuberculosis meningitis epidemic encephalitis anterior poliomyelitis and latent syphilis

The segmented cells outnumber the lymphocytes in the following diseases pneumococcus meningitis influenzal meningitis pyogenic meningitis and epidemic meningitis

The procedure for the differential cell count follows

And

average count × correction factors = eosinophils per cu mm

9 × 11 = 99

## SPINAL CELL COUNT

### Information Significant to the Student

The cells found in spinal fluid are usually white cells Sometimes however red cells produced by the puncture it self are seen These may cause a slight increase in the count

The normal values for the spinal cell count are 0 to 10 cells per cubic millimeter Increased values such as 20 to 1000 cells may be found in meningitis encephalitis poliomyelitis and latent syphilis

### Procedure for the Spinal Cell Count

Since the cells disintegrate rapidly the count should be made while the fluid is fresh—preferably within an hour after the withdrawal of fluid

Spinal fluid often contains contagious material Consequently the technician should take particular precautions in handling the specimens In making transfers of the fluid it is perhaps a wise policy to use medicine droppers rather than pipets

The spinal cell count consists of the total cell count and the differential cell count The procedures follow

#### Total Cell Count

1 In order to hemolyze any red cells that may be present add a few drops of 10% acetic acid to about 1 cubic centimeter of the spinal fluid Put a cork in the mouth of the tube and invert 8 to 10 times to mix
2 Using a medicine dropper transfer a small portion of this spinal fluid to a counting chamber which is generally used for counting blood cells

PLATE V


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1 Centrifuge the spinal fluid and discard the supernatant fluid
2 Place a small drop of the sediment on a glass slide Using another slide as a spreader spread the sediment over the slide as you would for a blood smear
3 Allow to dry
4 Stain with Wright's stain
5 Using the oil immersion objective of the microscope record the number of lymphocytes and segmented cells seen while counting 100 white cells
6 Report the percentage found
7 Example 40 lymphocytes and 60 segmented cells were recorded while counting 100 white cells Therefore there are 40% lymphocytes and 60% segmented cells

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LEGEND FOR PLATE V —*P falciparum*

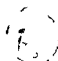
1 Very young ring form trophozoite
2 Double infection of single cell with young trophozoites one a marginal form the other signet ring form
3 4 Young trophozoites showing double chromatin dots
5 6 7 Developing trophozoite forms
8 Three medium trophozoites in one cell
9 Trophozoite showing pigment in a cell containing Maurer's spots
10 11 Two trophozoites in each of two cells showing variation of forms which they may assume
12 Almost mature trophozoite showing haze of pigment throughout cytoplasm Maurer's spots in the cell
13 Aestivo autumnal slender forms
14 Mature trophozoite showing clumped pigment
15 Parasites in the process of initial chromatin division
16 17 18 19 Various phases of the development of the schizont ("presegmented schizonts )
20 Mature schizont
21 22 23 24 Successive forms in the development of the gametocyte —usually not found in the peripheral circulation
25 Immature macrogametocyte
26 Mature macrogametocyte
27 Immature microgametocyte
28 Mature microgametocyte

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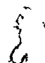

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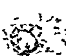

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## LEGEND FOR PLATE VI —*P. vivax*

1 Normal sized red cell with marginal ring form trophozoite
2 Young signet ring form trophozoite in a macrocyte
3 Slightly older ring form trophozoite in red cell showing basophilic stippling
4 Polychromatophilic red cell containing young tertian parasite with pseudopodia
5 Ring form trophozoite showing pigment in cytoplasm in an enlarged cell containing Schuffner's stippling
6 7 *Very tenuous medium trophozoite forms*
8 Three ameboid trophozoites with fused cytoplasm
9 11 12 13 Older ameboid trophozoites in process of development
10 Two ameboid trophozoites in one cell
14 Mature trophozoite
15 Mature trophozoite with chromatin apparently in process of division
16 17 18 19 Schizonts showing progressive steps in division (pre-segmenting schizonts)
20 Mature schizont
21 22 Developing gametocyte
23 Mature microgametocyte
24 Mature macrogametocyte

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Schuffner's stippling does not appear in all cells containing the growing and older of *P. vivax* as would be indicated by this picture but it can be found with any stain from the fairly young ring form onward

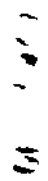
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## Legend for Plate VII—*P. malariae*

1. Young ring form trophozoite of quartan malaria.
2, 3, 4. Young trophozoite forms of the parasite showing gradual increase of chromatin and cytoplasm.
5. Developing ring form trophozoite showing pigment granule.
6. Early band form trophozoite—elongated chromatin, some pigment apparent.
7, 8, 9, 10, 11, 12. Some forms which the developing trophozoite of quartan may take.
13, 14. Mature trophozoites—one a band form.
15, 16, 17, 18, 19. Phases in the development of the schizont (presegmenting schizonts).
20. Mature schizont.
21. Immature microgametocyte.
22. Immature macrogametocyte.
23. Mature microgametocyte.
24. Mature macrogametocyte.

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# MALARIA PARASITES

**Information Significant to the Student**

Malaria is a parasitic disease which is transmitted to man by means of a mosquito The parasites live in the blood stream and go through a stage of development which is known as schizogony—meaning sexless They repay their host—man—by entering his red cells and causing hemolysis *This produces a toxicity which is accompanied by chills and fever* The treatment consists of administering drugs that are injurious to the parasite

The diagnosis of malaria depends largely upon finding the parasites in the blood stream There are 3 main species of the parasite Plasmodium vivax Plasmodium malariæ and Plasmodium falciparum These species are illustrated in Plates V VI and VII

**Examination for Malaria Parasites**

If the patient is not having an attack it is difficult to detect malaria parasites *Consequently it is strongly urged* that the patient be in the chills and fever stage at the time the blood is withdrawn

There are two methods of examination for malaria parasites the thin smear method and the thick smear method

The thin smear method consists of making a blood smear in the usual manner staining it with Wright's stain and examining the red cells for parasites This method is recommended for beginners

The thick smear method concentrates the cells and thus makes it possible to examine more cells in a shorter period of time This procedure consists of placing a drop of blood on a slide staining it in such a manner that the red cells are hemolyzed and then examining the slide for parasites This method is recommended for those with previous experience

The procedures for the thin smear method and the thick smear method are given below

**Thin Smear Method**

1 *Get materials for a finger puncture and a few glass slides*

7 Stain for 30 minutes
8 Remove from staining dish and dip in buffer solution to rinse
9 Dry in air
10 Using the oil immersion objective examine for malaria parasites The red cells of course will be hemolized But the malaria parasites if present will be more concentrated and will stand out much better than in the thin smear method
11 Report the presence or absence of malaria parasites

## BONE MARROW SMEARS

### Information Significant to the Student

The bone marrow is the site of the cell factories Here the white cells red cells and platelets are being manufactured for their entrance into the blood stream If the mechanics of manufacture are faulty some on the spot information may be necessary This is furnished by a study of bone marrow smears

Bone marrow smears are of particular interest in the following diseases aplastic anemia pernicious anemia leukemia multiple myeloma malignant neutropenia Gaucher s disease thrombocytopenic purpura and metastases from carcinoma

The role played by the average technician consists of assisting the physician making the smears and staining the slides Some technicians with more advanced training may be allowed to evaluate their preparations

### Preparation of Bone Marrow Smears

The equipment for the bone marrow puncture is furnished by the physician The technician furnishes about 10 extra clean glass slides and materials for a finger puncture

The physician prepares the patient makes a puncture of the bone (usually the sternum) and withdraws about 0 3 cc of bone marrow This is passed to the technician who should proceed as follows

2 Make a finger puncture on the patient
3 Prepare two blood smears as you would for a differential white cell count Take care however to see that there is a definite thin portion to the smears This is necessary because you will be examining individual red cells and they must be spread out
4 Select the best smear and stain with Wright s stain
5 Examine with the oil immersion objective of the microscope Malaria parasites are illustrated in Plates X XI and XII The student should guard against mistaking platelets for parasites Mistaking platelets for parasites is a frequent source of error for the beginner
6 If parasites are present make the report as follows Malaria parasites present If parasites are not found after a 15 minute search make the report as follows No malaria parasites seen

#### Thick Smear Method

1 Get materials for a finger puncture and a few glass slide
2 Make a finger puncture on the patient
3 Place a large drop of blood on a glass slide Using the corner of another slide spread the blood around so that it covers an area about the size of a dime
4 Allow to dry
5 Make a fresh batch of Giemsa stain by placing 2 cc of the *stock* Giemsa stain in a container and adding 50 cc of buffer solution
(Directions for preparing the stock Giemsa stain and buffer solution come with the bottle of Giemsa stain )
6 Place the thick smear in a vertical position in a staining dish Cover with the fresh batch of Giemsa stain

## PLATE VIII



NORMAL BONE MARROW

Myeloid-erythroid ratio 3 or 4 to 1

*From Animated Hematology, courtesy of The Armour Laboratories*

1. Place a small amount of bone marrow on a glass slide. Make a smear as you would for a blood smear.
2. Make about 5 more smears.
3. Label the smears with the patient's name and date.
4. Make a finger puncture and prepare 2 blood smears for a differential white cell count. Label the smears with the patient's name and date. These smears are taken for the purpose of correlating the findings in the bone marrow with the findings in the circulating blood.
5. Allow the blood and bone marrow smears to dry.
6. Stain with Wright's stain.
7. Refer the smears to the physician for evaluation and diagnosis.

*Table 19.—Normal Values for Bone Marrow Smears*

| Cell | Normal Values in Per Cent |
|---|---|
| WHITE CELLS | |
| Myeloblasts | 0 to 5 |
| Progranulocytes | 2 to 8 |
| Neutrophilic myelocytes | 4 to 16 |
| Neutrophilic metamyelocyte | 5 to 20 |
| Neutrophilic band cell | 10 to 35 |
| Neutrophilic segmented cell | 7 to 30 |
| Eosinophilic cells | 1 to 4 |
| Basophilic cells | 0 to 1 |
| Lymphocytes | 5 to 15 |
| Monocytes | 0 to [illegible] |
| Plasmacytes | 0 to 1 |
| RED CELLS | |
| Rubriblasts | 0 to 1 |
| Prorubricytes | 1 to 4 |
| Rubricytes | 3 to 10 |
| Metarubricytes | 5 to 2 |
| PLATELETS | |
| Megakaryocytes | 0 to 3 |

8. The normal values for a differential cell count of the bone marrow are given in Table 19. An illustration of normal bone marrow smears is given in Plate VIII.

# HETEROPHILE ANTIBODY TEST

**Information Significant to the Student**

This is a test for infectious mononucleosis. In this disease the serum contains an antibody which will clump the red cells of sheep. Such an antibody is called a heterophile antibody.

If the heterophile antibody is present, however, it does not necessarily mean that the patient has infectious mononucleosis. This antibody is also present in people who have serum sickness and people who have recently received horse serum as an antitoxin.

In order to differentiate between the above diseases the heterophile antibody test is divided into two phases: The Presumptive Test and the Differential Test.

The Presumptive Test is run on all suspected cases. If this is negative, no further test is necessary. If this is positive, however, the Differential Test is run.

**Procedure for the Heterophile Antibody Test**

The procedure for the Presumptive Test and the Differential Test are given below.

**Presumptive Test**

The reagents needed are normal (0.85%) saline and sheep cells. The latter may be obtained through your local medical supply house.

1. Get materials for a venipuncture and a test tube. Make a venipuncture, withdraw about 5 cc of blood, and transfer to the test tube. Allow to clot and centrifuge to obtain the serum.

10 Using Table 20 report the test tube numbers and titers (dilution of serum) which show any clumping of cells For example if there is clumping in tubes 1 2 and 3 make the report as follows

*Report of Presumptive Test*

| | | | |
|---|---|---|---|
| Clumping in tubes | #1 | #2 | #3 |
| Clumping in titers | 1 7 | 1 14 | 1 28 |

11 In infectious mononucleosis there is usually clumping in at least the first 5 tubes that is through titer 1 112 If there is clumping in these 5 tubes the following Differential Test should be run

12 NOTE It should be mentioned that the heterophile antibodies are not present throughout the course of infectious mononucleosis Consequently if the clinical symptoms persist the heterophile antibody test should be repeated in a week or ten days

***Table 20 —Serum Dilutions in the Heterophile Antibody Test***

| *Test Tube Number* | *Titer (Dilution of Serum)* |
|---|---|
| 1 | 1 7 |
| 2 | 1 14 |
| 3 | 1 [illegible]8 |
| 4 | 1 56 |
| 5 | 1 112 |
| 6 | 1 224 |
| 7 | 1 448 |
| 8 | 1 896 |
| 9 | 1 1 792 |
| 10 | 1 3 584 |
| 11 | 1 7 168 |

13

2. Inactivate the serum at 56° C for 30 minutes
3. Get 12 small test tubes and a test tube rack. Label the tubes 1 to 12 and place in the rack. Mark the last tube *Control*
4. Place 0.40 cc of normal saline in test tube number 1 and 0.25 cc in the remaining tubes
5. Make a serial dilution of the serum and normal saline as follows: Add 0.1 cc of the inactivated serum to tube 1. Mix the contents of tube 1 and transfer 0.25 cc of the mixture to tube 2. Mix the contents of tube 2 and transfer 0.25 cc of the mixture to tube 3. Continue this dilution process through tube 11, adding nothing to tube 12 (the control). Now remove 0.25 cc from tube 11 and discard
6. Prepare a 2% saline suspension of the sheep cells as follows: Mix the sheep cells by inverting the container. Remove 0.1 cc of the cells from the container. Place in a test tube. Wash the cells 3 times by adding about 5 cc of normal saline, centrifuging and pouring off the saline. After the 3rd washing add 5 cc of normal saline. Mix gently
7. Add 0.1 cc of the above saline suspension of sheep cells to each of the 12 tubes. Mix gently. (Save the suspension of sheep cells as it will be used if the Differential Test is necessary.)
8. Let the tubes stand at room temperature for 2 hours
9. Gently shake the tubes to mix. Place the contents of tube 1 on a glass slide. Using the low power (16 mm.) objective of the microscope examine for clumping. In a similar manner examine the remaining tubes for clumping. Record your results. There should of course be no clumping in the control tube (number 12)

10 Using Table 20 report the test tube numbers and titers (dilution of serum) which show any clumping of cells For example if there is clumping in tubes 1 2 and 3 make the report as follows

*Report of Presumptive Test*

| | | | |
|---|---|---|---|
| Clumping in tubes | #1 | #2 | #3 |
| Clumping in titers | 1:7 | 1:14 | 1:28 |

11 In infectious mononucleosis there is usually clumping in at least the first 5 tubes that is through titer 1:112 If there is clumping in these 5 tubes the following Differential Test should be run

12 NOTE It should be mentioned that the heterophile antibodies are not present throughout the course of infectious mononucleosis Consequently if the clinical symptoms persist the heterophile antibody test should be repeated in a week or ten days

### *Table 20 — Serum Dilutions in the Heterophile Antibody Test*

| *Test Tube Number* | *Titer (Dilution of Serum)* |
|---|---|
| 1 | 1:7 |
| 2 | 1:14 |
| 3 | 1:28 |
| 4 | 1:[illegible] |
| 5 | 1:112 |
| 6 | 1:224 |
| 7 | 1:448 |
| 8 | 1:896 |
| 9 | 1:1[illegible] |
| 10 | 1:3,584 |
| 11 | 1:[illegible] |

**Differential Test**

It has been found that guinea pig kidney antigen will absorb the antibodies in the serum of the following: (1) people who are normal, (2) people who have serum sickness, and (3) people who have recently received an injection of horse serum. This guinea pig kidney antigen, however, will not absorb the antibodies in the serum of people with infectious mononucleosis. Consequently, the antigen can be used to distinguish between people who do *not* have infectious mononucleosis and people who do have infectious mononucleosis.

The following reagents are needed for the Differential Test: normal (0.85%) saline, sheep cells, and guinea pig kidney antigen. The sheep cells and antigen may be obtained through your local medical supply house.

1. Get 10 test tubes and a test tube rack. Number the tubes 1 to 10 and place in the rack.
2. Pipet 0.25 cc of normal saline into tubes 2 through 10.
3. Shake the guinea pig kidney antigen in order to mix.
4. Place 1.0 cc of the antigen in a small test tube.
5. Add 0.2 cc of the patient's inactivated serum.
6. Mix well and allow to stand 3 minutes.
7. Centrifuge. The supernatant fluid will contain the absorbed serum.
8. Carefully pipet off at least 0.5 cc of the supernatant fluid. Place in a small test tube.
9. Pipet 0.25 cc of the above supernatant fluid into tube 1. Also pipet 0.25 cc into tube 2.
10. Mix the contents of tube 2 and transfer 0.25 cc to tube 3.
11. Mix the contents of tube 3 and transfer 0.25 cc to tube 4.
12. Continue the above dilution process through tube 10. Now remove 0.25 cc from tube 10 and discard.

13 To each tube add 0 1 cc of the 2% saline suspension of sheep cells

14 Gently shake the test tube rack in order to mix

15 Let stand for 2 hours at room temperature

16 Gently shake the tubes to mix Place the contents of tube 1 on a glass slide Using the low power (16 mm) objective of the microscope examine for clumping In a similar manner examine the remaining tubes for clumping Record your results

17 Using Table 20 report the test tube numbers and titers which show clumping of cells For example if there is clumping in the first 3 tubes make the report as follows

*Report of Differential Test*

| | | | |
|---|---|---|---|
| Clumping in tubes | #1 | #2 | #3 |
| Clumping in titers | 1 7 | 1 14 | 1 28 |

18 In infectious mononucleosis clumping occurs in at least the first 3 tubes that is through titer 1 28

19 NOTE The above test may be confirmed by another absorption test with beef cell antigen This test however is seldom necessary It is run exactly like the above test except that the guinea pig kidney antigen is replaced by beef cell antigen The beef cell antigen will absorb the antibodies in the serum of people with infectious mononucleosis serum sickness and horse serum sensitization This serum will then be unable to clump the sheep cells

Chapter 7

# Blood Picture in Various Blood Diseases

The information which the technician gathers for the physician forms a pattern which is known as the patient s blood picture It is extremely useful in diagnosis For example if the physician has a diagnostic problem and speculation points to a disease of the blood a likeness is sought between the patient s blood picture and that seen in various blood diseases When such a correlation is found it may serve as the missing piece in the jig saw puzzle of diagnosis

This chapter presents the blood picture seen in the more common blood diseases They will be considered in the following order

- The Anemias
- The Leukemias
- Hemorrhagic Diseases
- Miscellaneous Diseases

## THE ANEMIAS

Anemia means lack of blood It is a condition characterized by *a reduced hemoglobin low red cell count or a combination of the two* These shortages may be caused by a *decrease in blood production increase in blood destruction* or a severe loss of blood For example if the cell factories *do not receive the proper raw materials* the production of red cells is decreased and a deficiency anemia results If the *factories go on strike production goes down* and an aplastic anemia follows Should the cells leave the factories and *become highjacked somewhere in the circulatory system* increased blood destruction occurs and a hemolytic anemia results Finally an injury or operation may cause a severe loss of blood and a posthemorrhagic anemia follows

The anemias which will be discussed under the above classification are given below

- I Decreased blood production
  - A Deficiency anemias
    - 1 Pernicious anemia
    - 2 Simple achlorhydric anemia
  - B Aplastic anemias
    - 1 Aplastic anemia
- II Increased blood destruction
  - A Hemolytic anemias
    - 1 Sickle cell anemia
    - 2 Congenital hemolytic anemia
- III Severe blood loss
  - A Posthemorrhagic anemias
    - 1 Acute posthemorrhagic anemia

In the laboratory the anemias are discovered by finding a low hemoglobin or a low red cell count Sometimes however both the hemoglobin and red cell count are low

The hemoglobin content and size of the red cells associated with the anemias give rise to a further classification which the student will find useful since it offers a description of the cells involved For instance if the cells are smaller than normal they are called microcytes cells normal in size are referred to as normocytes and cells larger than normal are called macrocytes When the cells are deficient in hemoglobin they are labeled hypochromic cell having the normal quota of hemoglobin are called normochromic and cells having an increased amount of hemoglobin are referred to as hyperchromic Thus when classified descriptively the following nine anemias are theoretically possible

| | | |
|---|---|---|
| I Microcytic | hypochromic<br>normochromic<br>hyperchromic | anemia |
| II Normocytic | hypochromic<br>normochromic<br>hyperchromic | anemia |
| III Macrocytic | hypochromic<br>normochromic<br>hyperchromic | anemia |

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The information which the technician gathers for the physician forms a pattern which is known as the patient's blood picture. It is extremely useful in diagnosis. For example, if the physician has a diagnostic problem and speculation points to a disease of the blood, a likeness is sought between the patient's blood picture and that seen in various blood diseases. When such a correlation is found, it may serve as the missing piece in the jig-saw puzzle of diagnosis.

This chapter presents the blood picture seen in the more common blood diseases. They will be considered in the following order:

The Anemias
The Leukemias
Hemorrhagic Diseases
Miscellaneous Diseases

## THE ANEMIAS

Anemia means lack of blood. It is a condition characterized by a reduced hemoglobin, low red cell count, or a combination of the two. These shortages may be caused by a decrease in blood production, increase in blood destruction, or a severe loss of blood. For example, if the cell factories do not receive the proper raw materials, the production of red cells is decreased and a deficiency anemia results. If the factories go on strike, production goes down and an aplastic anemia follows. Should the cells leave the factories and become highjacked somewhere in the circulatory system, increased blood destruction occurs and a hemolytic anemia results. Finally, an injury or operation may cause a severe loss of blood and a posthemorrhagic anemia follows.

large and well packed with hemoglobin consequently the color index and volume index are high The blood slide usually shows hypersegmented neutrophils nucleated red cells and greatly enlarged normochromic erythrocytes Anisocytosis poikilocytosis and polychromatophilia are quite marked

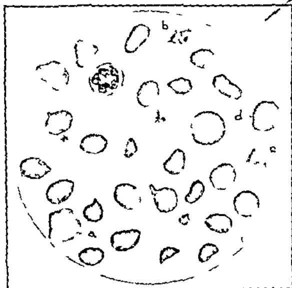



Fig 65 – Drawing of blood smear from a case of pernicious anemia in relapse Note the hypersegmented neutrophil and the large normochromic red cell Anisocytosis and poikilocytosis are quite marked Cell (a) is a large polychromatophilic red cell Cell (b) contains a Howell Jolly body Cell (c) contains a Cabot ring and cell (d) is a granule red capsule (Wintrobe *Clinical Hematology*)

After treatment the red cell production is greatly increased the reticulocyte count goes up and the blood picture returns to normal A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Figure 65 (Other laboratory findings in addition to the blood picture are a high icteric index and the absence of free hydrochloric acid in the stomach )

Blood pictures of the individual anemias, beginning with pernicious anemia are given on the following pages The cells in all the illustrations have been stained with Wright s stain

## PERNICIOUS ANEMIA

(*Synonyms* Addisonian anemia Biermer s anemia)

Pernicious anemia is a deficiency anemia confined mainly to the middle aged It is characterized by weakness stomach disturbances and sore tongue Until recent years diagnosis of the disease was synonymous with a death warrant However in 1926 Minot and Murphy made the epoch making discovery that the disease could be relieved by the administration of liver For this piece of research they received the Nobel Prize

*Table 21 —Typical Blood Picture in Pernicious Anemia*

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1 to 3 million | 4 0 to 5 5 million |
| Hemoglobin | 4 to 11 Gm | 12 to 17 Gm |
| Color index | 1 20 to 1 60 | 0 90 to 1 10 |
| Volume index | 1 10 to 1 50 | 0 90 to 1 10 |
| Blood slide | White cell abnormalities<br>Hypersegmented neutrophils | |
| | Red cell abnormalitie<br>Nucleated red cells large normochromic red cells anisocytosis poikilocytosi and polychromatophilia | |

The blood picture before treatment shows a low hemoglobin and low red cell count The white cell and platelet counts are also below normal The red cells are unusually

index is low A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Figure 66

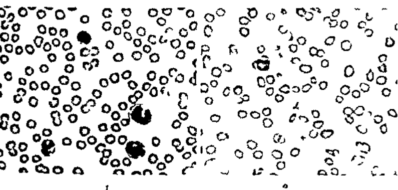


FIG 66 —Blood slides illustrating normal and hypochromic erythrocytes (from Haden) *1* Normal blood *2* hypochromic erythrocytes Note the presence of small hypochromic erythrocytes Increased variations in the shape of the erythrocytes (poikilocytosis) is moderate

## SIMPLE ACHLORHYDRIC ANEMIA

(*Synonyms*: Idiopathic hypochromic anemia, chronic hypochromic anemia, late chlorosis)

Simple achlorhydric anemia is an iron deficiency anemia which is usually confined to women. The name of the disease is derived from the absence of free hydrochloric acid (achlorhydria) in the gastric juices of patients having the disease. This anemia is characterized by weakness, menstrual disturbances, and a greenish coloration of the skin. Patients suffering from this type of anemia are relieved by the administration of large doses of iron.

***Table 22.—Typical Blood Picture in Simple Achlorhydric Anemia***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1 to 4 million | 4.0 to 5.5 million |
| Hemoglobin | 4 to 10 Gm. | 12 to 17 Gm. |
| Color index | 0.50 to 0.80 | 0.90 to 1.10 |
| Blood slide | White cell abnormalities: None | |
| | Red cell abnormalities: Many small hypochromic erythrocytes; poikilocytosis is moderate | |

The blood picture shows a reduced hemoglobin and low red cell count. The stained red cells of the blood slide are small and markedly pale due to a shortage of hemoglobin. Often the hemoglobin is so deficient that the cells look like hollow rings. Since the cells are lacking in hemoglobin, the color

index is low A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Figure 66

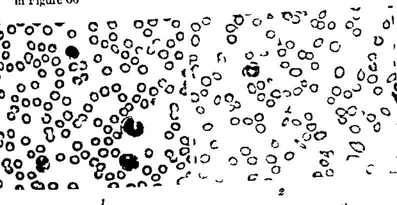



Fig 66 —Blood slides illustrating normal and hypochromic erythrocytes (from Haden) *1* Normal blood *2* hypochromic erythrocytes Note the presence of small hypochromic erythrocytes Increased variations in the shape of th erythrocytes (poikilocytosis) is moderate

# APLASTIC ANEMIA

(*Synonym* Bone marrow insufficiency)

Aplastic anemia is a rare disease of the blood which may be caused by exposure to radioactive rays x rays and poisoning with benzol or arsenic compounds Often the cause is not apparent It is characterized by weakness a waxy pallor of the skin and bleeding from the mucous membranes Patients having the disease generally die within six months

***Table 23 —Typical Blood Picture in Aplastic Anemia***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Red cell count | 0 5 to 2 5 million | 4 0 to 5 5 million |
| Hemoglobin | 1 to 7 Gm | 12 to 17 Gm |
| White cell count | 1 000 to 3 000 per cu mm | 5 000 to 10 000 per cu mm |
| Platelet count | 5 000 to 50 000 per cu mm | 250 000 to 350 000 per cu mm |
| Blood slide | White cell abnormalities None | |
| | Red cell abnormalities Vital stain of blood slide shows a low reticulocyte count | |

The blood picture shows a greatly reduced hemoglobin and very low red cell count The white cells and platelets are also reduced in number The cells of the blood slide are normal but the reticulocyte count made after a vital stain is extremely low A typical blood picture is given in the accompanying table and the bleeding gums found in a case of aplastic anemia are illustrated in Figure 67

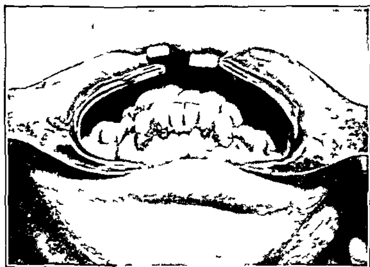


Fig. 67.—Bleeding gums in a case of aplastic anemia
(Wintrobe, *Clinical Hematology*.)

## SICKLE CELL ANEMIA

(*Synonyms*: Sicklemia, Herrick's syndrome)

Sickle cell anemia is a hemolytic anemia which is confined almost exclusively to members of the negro race. Its cause is unknown. The disease is hereditary and roughly speaking is transmitted to five out of every thousand negroes. Patients having this disease generally die at an early age. J. W. Diggs, who made a special study of sickle cell anemia, found that the average age at death was 11 years.

***Table 24.—Typical Blood Picture in Sickle Cell Anemia***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1 to 4 million | 4.0 to 5.5 million |
| Hemoglobin | 3 to 12 Gm. | 12 to 17 Gm. |
| Sickle cell examination | Presence of many sickle-shaped red cells | |

The blood picture shows a reduced hemoglobin and low red cell count. The demonstration of sickle-shaped red cells is the most significant feature in the blood picture. It is emphasized by Kracke, however, that sickle-shaped red cells are present in many individuals who never come down with the disease. A typical blood picture is given in the accompanying table and sickle-shaped red cells are illustrated in Figure 68.

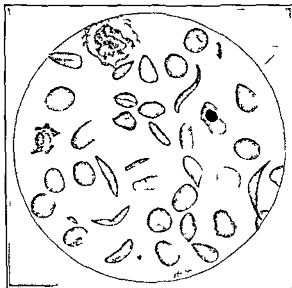


Fig 68 —Drawing of a blood smear from a case of sickle cell anemia Note the presence of many sickle-shaped red cells (Wintrobe *Clinical Hematology*)

## CONGENITAL HEMOLYTIC ANEMIA

*(Synonyms* Congenital hemolytic icterus, Spherocytic jaundice)

Congenital hemolytic anemia is an inherited disease which is characterized by relapses and remissions During the remissions patients are apparently in good health but during relapses they may become jaundiced or show other signs of anemia The clinical symptoms of this disease are too generalized for diagnostic purposes but the laboratory findings are extremely significant to the physician

***Table 25 —Typical Blood Picture in Congenital Hemolytic Anemia During a Relapse***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1.5 to 3.0 million | 4.0 to 5.5 million |
| Hemoglobin | 4 to 8 Gm | 12 to 1[illegible] Gm |
| Fragility test | hem begins 0.50%<br>hem end 0.40% | hem begins 0.44%<br>hem ends 0.34% |
| Reticulocyte count | 10 to 30% | 1 to 2% |
| Blood slide | White cell abnormalities Many metamyelocytes and occasional myelocyte | |
| | Red cell abnormalities Spherocytes and metarubricytes are present *anisocytosis* poikilocytosis and polychromatophilia are marked | |

The blood picture during remissions is usually normal However during relapses the hemoglobin drops and the red cell count falls A decided increase in the hemolysis of red cells causes the icteric index to rise The body attempts to

compensate for the increased destruction of cells and pours immature cells into the circulation Consequently the reticulocyte count goes up and metarubricytes appear in the blood slide Anisocytosis poikilocytosis and polychromatophilia are marked

The most significant feature of the blood slide is the appearance of small heavily stained red cells called spherocytes The connection between the spherocytes and the disease is stated by Haden The fundamental difficulty in this disease is an anatomic defect in the shape of the red cells which are spherocytes rather than normal biconcave disks As a result of this abnormal shape the cells are more fragile than normal and are rapidly removed from the circulation Consequently when a red cell fragility test is performed the red cells show increased fragility A typical blood picture of this disease is given in the accompanying table and the spherocytes and reticulocytes found in the blood slide are illustrated in Figure 69

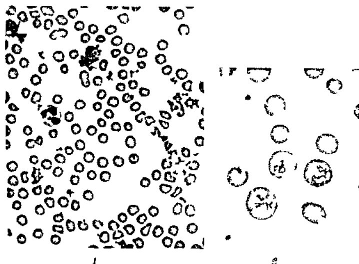



FIG 69 —Illustration of spherocytes and reticulocytes 1 Spherocyte Reticulocytes stained with a vital stain (Haden *Principles of Hematology*)

## ACUTE POSTHEMORRHAGIC ANEMIA

Following injury or operation a person may lose a considerable amount of blood. The decreased volume of blood could result in the condition known as shock. This can be prevented, however, by the administration of plasma or other fluids. When such treatment is necessary, the blood becomes diluted and shows signs of an anemia which is referred to as posthemorrhagic anemia.

*Table 26.—Typical Blood Picture in Acute Posthemorrhagic Anemia*

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1 to 4 million per cu. mm. | 4.0 to 5.5 million per cu. mm. |
| Hemoglobin | 3 to 11 Gm. | 12 to 17 Gm. |
| Color index | 0.40 to 0.80 | 0.90 to 1.10 |
| Volume index | 0.40 to 0.80 | 0.90 to 1.10 |
| Blood slide | White cell abnormalities: None | |
| | Red cell abnormalities: Many hypochromic microcytes, occasional metarubricyte | |

The blood picture immediately following the administration of fluids shows a reduced hemoglobin and low red cell count. This is caused by the dilution of the blood. The body endeavors to compensate for the loss of blood and produces immature cells at a rapid rate. Young cells often deficient in hemoglobin are hurried into the circulation. Consequently, the blood slide may show small red cells which are lacking in the normal quota of hemoglobin. These cells are called hypochromic microcytes. An occasional metarubricyte

may be found and the reticulocyte count is increased Polychromatophilic cells are often present The above condition may last for about 10 days and then the blood picture re

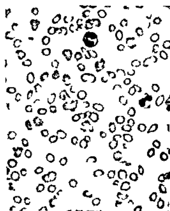


Fig 70 —Hypochromic microcytes
(Haden *Principles of Hematology* )

turns to normal A typical blood picture during the anemia is given in the accompanying table and the characteristic hypochromic microcytes found in the blood slide are illustrated in Figure 70

## THE LEUKEMIAS

Leukemia is a disease of the blood forming tissues which is characterized by a tremendous increase in the production of white cells. The disease is rare, its cause is unknown and it is usually fatal. The classification of the leukemias is based on the course of the disease and the predominating type of white cells. Thus there are both acute and chronic granulocytic, lymphocytic, monocytic and plasmacytic leukemias (Table 27).

*Table 27.—Classification of the Leukemias*

| | |
|---|---|
| 1. Granulocytic leukemia | 3. Monocytic leukemia |
| *a.* Acute | *a.* Acute |
| *b.* Chronic | *b.* Chronic |
| 2. Lymphocytic leukemia | 4. Plasmacytic leukemia |
| *a.* Acute | *a.* Acute |
| *b.* Chronic | *b.* Chronic |

Patients having any of the acute leukemias usually die within several months. A great rise in the white cell count occurs and many immature cells appear in the blood slide. Quite often the cells are so immature that it is difficult to tell whether they belong to the granulocytic, lymphocytic, monocytic, or plasmacytic series. Consequently the exact diagnosis of the disease often becomes difficult. Sometimes the company they keep furnishes a valuable clue. For example, if the *mature* lymphocytes outnumber the *mature* granulocytes, the chances are good that the young forms belong to the lymphocytic series.

Another means of differentiating immature members of the granulocytic and lymphocytic series is the peroxidase stain. The peroxidase stain demonstrates the presence of an oxidizing ferment in the cytoplasm of the granular series of cells with the exception of the myeloblast which reacts negatively since it alone contains no granules. (Kracke.) The cells of the granulocytic series, with the exception of the

myeloblasts are therefore peroxidase positive whereas the cells of the lymphocytic series are peroxidase negative

The procedure for the peroxidase stain is as follows

**Peroxidase Stain (Sato and Sekiya)**

1 Preparation of solutions
   *a* 0 5% aqueous solution of copper sulfate Using the analytical balance weigh out 0 500 Gm of anhydrous copper sulfate Place in a 100 cc volumetric flask and add distilled water to the 100 cc mark Mix
   *b* Benzidine solution Using the rough balance weigh out 0 1 Gm of benzidine Place in 100 cc of distilled water Mix and filter *Just before use* add a few drops of hydrogen peroxide
   *c* 1% aqueous solution of safranin Using the rough balance weigh out 1 Gm of safranin Place in a container and add 99 cc of distilled water Mix
2 Make a blood slide
3 Cover the slide with the 0 5% aqueous solution of copper sulfate Let stand for about 25 seconds Pour off the copper sulfate solution and do *not* wash
4 Cover the blood slide with the benzidine solution Let stand for 5 minutes Pour off the benzidine solution and do *not* wash
5 Cover the blood slide with the 1% aqueous solution of safranin Let stand for 2 minutes
6 Wash dry and examine with the oil immersion objective The granules in the cells of the granulocytic series (with the exception of the myeloblasts) stain a deep purple The cells of the lymphocytic series since they lack the granules do not take the stain The cells of the monocytic series may or may not take the stain

In the chronic leukemias the white cell count often rises to ten or twenty times the normal values and numerous immature cells appear in the blood slide For instance in granulocytic leukemia it is not uncommon to see a white cell count of 200 000 and find 35 myelocytes in the differential It is possible for a remission to occur and in such cases the white cell count may return to normal or even drop below normal Unfortunately remissions are not permanent and a return to the typical blood picture follows Patients having any of the chronic leukemias generally die within four years No successful treatment has yet been found but frequent transfusions and x ray therapy may prolong the patient s life

Since plasmacytic leukemia is extremely rare and the acute leukemias are seldom met by the technician the only forms of the disease which will be considered are chronic granulocytic leukemia chronic lymphocytic leukemia and chronic monocytic leukemia The blood pictures follow The cells in the illustrations have been stained with Wright s stain

# PLATE IX

Eosinophilic myelocyte

Neutrophilic myelocyte

Neutrophilic metamyelocyte

Normoblast (metarubricyte)

Progranulocyte

Neutrophilic segmented cell

Neutrophilic band cell

Neutrophilic myelocyte

Neutrophilic band cell

Myeloblast

Neutrophilic metamyelocyte

Neutrophilic myelocyte

Neutrophilic segmented cell

Neutrophilic band cell

BLOOD SLIDE IN CHRONIC GRANULOCYTIC LEUKEMIA

## CHRONIC GRANULOCYTIC LEUKEMIA

Chronic granulocytic leukemia is a rare disease of the blood which is generally confined to adults. It is characterized by an insidious onset, weakness and emaciation. Patients having the disease usually die within four years.

***Table 28.—Typical Blood Picture in Chronic Granulocytic Leukemia***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| White cell count | 50 000 to 100 000 per cu mm | 5 000 to 10 000 per cu mm |
| Blood slide | White cell abnormalities: 5 to 35% myelocytes, occasional promyelocyte and myeloblast. Metamyelocyte and band cells present. | |
| | Red cell abnormalities: 1 to 4% metarubricytes; mature red cells show anisocytosis, poikilocytosis and polychromatophilia. | |

The blood picture shows a greatly increased white cell count with immature cells, both red and white, appearing in the blood slide. Following an acute infection or during treatment with x rays the white cell count may return to normal. Immature white cells, however, continue to appear in the differential white cell count. A typical blood picture of this disease is given in the accompanying table and the cells of the blood slide are illustrated in Plate IX.

## CHRONIC LYMPHOCYTIC LEUKEMIA

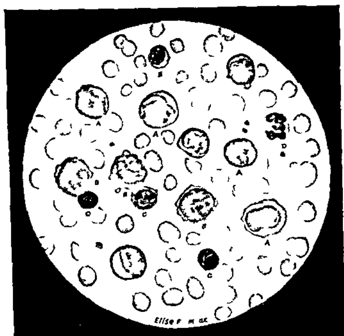
Chronic lymphocytic leukemia is a rare disease of the blood which is generally confined to adults. The cause of the disease is unknown and it is usually fatal. Life may be prolonged by transfusions but patients having this disease usually die within four years. The most significant clinical finding is a swelling of the lymph glands.

*Table 29.—Typical Blood Picture in Chronic Lymphocytic Leukemia*

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| White cell count | 40 000 to 80 000 per cu. mm. | 5 000 to 10 000 per cu. mm. |
| Blood slide | White cell abnormalities: Many lymphocytes (80 to 95%), immature lymphocytes (lymphoblasts and prolymphocytes) are present. | |
| | Red cell abnormalities: Occasional metarubricyte, mature red cells show anisocytosis, poikilocytosis and polychromatophilia. | |

The blood picture shows a tremendous increase in the white cell count. The number of lymphocytes in the blood slide is greatly increased and many immature forms are present. A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Plate X.

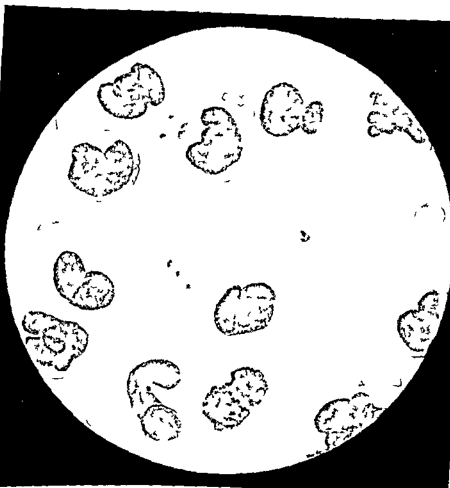
PLATE X




LYMPHOCYTIC LEUKEMIA

A Lymphoblast *B* [illegible] lymphoid cell *C* lymphocyte *D* neutrophil segmented cell *E* rubricyte (*W*[illegible] *be Tice Practice of Medicine* [illegible] *W. F.* [illegible] *Company*)

PLATE XI



CHRONIC MONOCYTIC LEUKEMIA

The cell nearest the center which lacks granules is a promonocyte. All the other cells are monocytes. (Kracke, *Diseases of the Blood*, 2nd edition. Courtesy of J. B. Lippincott Company.)

## CHRONIC MONOCYTIC LEUKEMIA

Chronic monocytic leukemia is a rare disease of the blood which is usually confined to adults It is characterized by a sudden onset weakness and emaciation The disease is fatal and generally terminates at an earlier stage than either granulocytic or lymphocytic leukemia One of the early clinical symptoms are oral disturbances these may include bleeding gums sore throat and swelling of the mucous membranes

*Table 30 —Typical Blood Picture in Chronic Monocytic Leukemia*

| *Sign Point Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| White cell count | 40 000 to 80 000 per cu mm | 5 000 to 10 000 per cu. mm |
| Blood slide | White cell abnormalities 60 to 95% monocytes | |
| | Red cell abnormalities Occasional metarubricyte mature red cells usually show anisocytosis poikilocytosis and polychromatophilia | |

The blood picture shows a very high white cell count with many monocytes appearing in the blood slide A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Plate XI

## HEMORRHAGIC DISEASES

Hemorrhagic diseases are characterized by abnormal bleeding The bleeding may be external such as bleeding from the nose and mucous membranes or it may be internal such as hemorrhages into the tissues and joints In some cases both external and internal bleeding are involved

Some hemorrhagic diseases are caused by a weakness in the capillary walls An example of this type is scurvy Other hemorrhagic diseases are caused by a shortage of materials needed for the blood clot Examples of this type are (1) essential thrombocytopenic purpura which is due to a shortage of thromboplastin and (2) hemorrhagic disease of the newborn which is due to a shortage of prothrombin

The hemorrhagic diseases which will be discussed are

1 Essential thrombocytopenic purpura
2 Hemophilia
3 Hemorrhagic disease of the newborn

# ESSENTIAL THROMBOCYTOPENIC PURPURA

(*Synonyms* Idiopathic thrombocytopenic purpura hemorrhagica, Werlhof s disease)

Essential thrombocytopenic purpura is characterized by both internal and external bleeding the external bleeding being from the mucous membranes The cause of the disease is unknown It is more common in children and young adults than in older people Quite frequently the only clinical symptoms are repeated nosebleeds prolonged menstruations and bleeding from the gums Transfusions tend to relieve the situation and the outlook for this disease is usually favorable

***Table 31 —Typical Blood Picture in Essential Thrombocytopenic Purpura***

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Bleeding time | 20 to 60 min | 1 to 3 min |
| Platelet count | 50 000 to 200 000 | 2 0 000 to 350 000 |
| Clot retraction time | no retraction in 24 hours | complete retraction in 24 hours |
| Capillary resistance test | positive | negative |

The laboratory findings are an increased bleeding time a decreased platelet count a prolonged clot retraction time and a positive capillary resistance test The coagulation time and prothrombin time are normal

A typical blood picture is given in the accompanying table and the abnormal clotting mechanism is illustrated in Figure 71

### Clotting Mechanism in Essential Thrombocytopenic Purpura

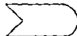
The meaning of the following 3 symbols is printed beneath each symbol. These symbols are then used in the illustration below.

CLOTTING MECHANISM IN THROMBOPENIA










[THROMBOPLASTIN]





[THROMBIN]

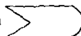
CA PROTHROMBIN





[CLOT]

FIBRINOGEN



Substances in parenthesi are not normally in plasma
Shad d substa es are def ent

Fig 71 —The clotting mechanism in essential thrombocytopenic purpura Here the defect is a quantitative deficiency in the thromboplastin due to the thrombopenia (Haden Principles of Hematology)

## HEMOPHILIA

Hemophilia is a rare disease of the blood which is characterized by excessive bleeding The bleeding may be internal or external The internal bleeding is frequently spontaneous and is marked by the seepage of blood into the tissues and joints of the body The external bleeding may result from a tooth extraction or injury to the skin

*Table 32 —Typical Blood Picture in Hemophilia*

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Coagulation time | 10 to 120 min | 2 to 6 min |

The disease is inherited and confined only to males A man having hemophilia can not pass the disease on to his sons, but his daughters may inherit the genetic trait and transmit the disease to their sons The majority of patients having hemophilia die before reaching manhood However if they do live to become an adult their chances of living a normal life span are good The usual treatment during a hemorrhage is complete bed rest and blood transfusions

The laboratory findings are an increased coagulation time The bleeding time is usually normal and the platelet count and clot retraction time are within normal values The capillary resistance test is negative A typical blood picture is given in the accompanying table and the clotting mechanism is illustrated in Figure 72

### Clotting Mechanism in Hemophilia

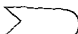
The meaning of the following 3 symbols is printed beneath each symbol. These symbols are then used in the illustration below.

**CLOTTING MECHANISM IN HEMOPHILIA**











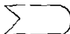











Substances in parenthesis not normally in plasma
Shaded substances are deficient

Fig. 72.—The clotting mechanism in hemophilia. Here the defect is in a qualitative abnormality of the platelets so thromboplastin is not normally released. (Haden: *Principles of Hematology.*)

# HEMORRH \GIC DISE \SF OF THE NEW BORN

This hemorrhagic disease of inf ints is characterized by bleeding into the intestinal tract beneath the surface of the skin or from the umbilical cord It is present in about 1 per cent of newborn infants (Todd and Sanford) The bleeding may occur anytime during the first two weeks of life A temporary deficiency in prothrombin accompanies the disease and the condition is relieved by blood transfusions

*Table 33 —Typical Blood Picture in Hemorrhagic Disease of the Newborn*

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Bleeding time | 6 to 12 min | 1 to 3 min |
| Coagulation time | 10 to 20 min | 2 to 6 min |
| Prothrombin time | 20 to 60 sec | 12 to 17 sec |

The laboratory tests show that the bleeding coagulation and prothrombin times are prolonged The platelet count and clot retraction time are normal The capillary resistance test is negative A typical blood picture is given in the accompanying table and the abnormal clotting mechanism is illustrated in Figure 73

## Clotting Mechanism in Hemorrhagic Diseases of the Newborn

The meaning of the following 3 symbols is printed beneath each symbol These symbols are then used in the illustration below

CLOTTING MECHANISM IN HEMORRHAGE OF THE NEW BORN

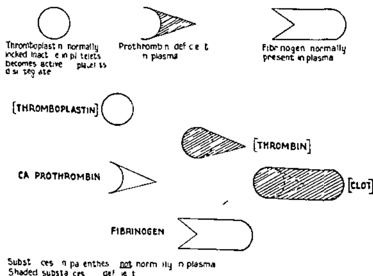



Fig 3 —The clotting mechanism in hemorrhagic disease of the newborn Here the defect is an insufficient supply of prothrombin (Haden *Principle of Hematology*)

## MISCELLANEOUS DISEASES

There are many diseases of the blood that cannot be properly classified under the anemias leukemias or hemorrhagic diseases For instance anemias are characterized by a low red cell count But is there a disease having a high red cell count? The leukemias have extremely high white cell counts But is there a disease having an unusually low white cell count?

Some of the more common blood disorders which cannot be grouped under the anemias leukemias or hemorrhagic diseases will be considered in this section The diseases which will be discussed are

1 Polycythemia vera
2 Malignant neutropenia
3 Erythroblastosis fetalis
4 Infectious mononucleosis

## POLYCYTHEMIA VERA

(*Synonym* Erythremia)

Polycythemia vera is a rare disease of the blood which is characterized by a tremendous increase in the red cell count It is generally confined to people over forty its cause is unknown and it is usually fatal The disease has an insidious onset and it is accompanied by weakness hemorrhages and a red coloration of the skin

Treatment consists of decreasing the red cell production and increasing the red cell destruction The production of red cells is decreased by radiation to the bone marrow and the red cell destruction is increased by the administration of chemicals which act as hemolytic agents

The blood picture shows a greatly increased red cell count and a very high hemoglobin The white cell count may be normal or increased The hematocrit reading and platelet count are high There are usually immature red and white cells in the blood slide The viscosity of the blood is greatly increased and the total volume of blood is above normal A typical blood picture is given in the accompanying table and a photograph of a patient with the disease is given in Figure 74

**Table 34 —Typical Blood Picture in Polycythemia Vera**

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Red cell count | " to 12 million per cu mm | 4 0 to 5 5 million per cu mm |
| Hemoglobin | 18 to 24 Gm | 12 to 1/ Gm |
| Hematocrit reading | 60 to 80% | 37 to 50% |
| Platelet count | 800 000 to 2 000 000 p r cu mm | 250 000 to 350 000 per cu mm |



FIG 74 —Photograph of a drawing (original in col r) of on of O l r's cases of erythremia (Courtesy of the Johns Hopkins Ho pital from Wintrobe *Clinical Hematology* )

## MALIGNANT NEUTROPENIA

(*Synonyms* Agranulocytosis Idiopathic neutropenia)

Malignant neutropenia is characterized by a marked decrease in the white cell count The majority of cases are due to the effect on the bone marrow of amidopyrine or related drugs in a drug sensitive person (Haden) Since compounds containing amidopyrine are frequently used in remedies for pain the disease may be curbed by the careful administration of these drugs The outlook for the chronic course of the disease is usually favorable however the decreased number of white cells may enable a slight infection to produce fatal results If the disease is acute death may come within a few days

*Table 35 —Typical Blood Picture in Malignant Neutropenia*

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| White cell count | 200 to 2 000 per cu mm | 5 000 to 10 000 per cu mm |
| Blood slide | White cell abnormalities Decreased percentage of neutrophilic cells Toxic granulation of neutrophilic cell | |
| | Red cell abnormalities None | |

The significant features of the blood picture are a low white cell count and toxic granulation in the neutrophilic cells of the blood smear The toxic granulation appears as a coarse heavy precipitate in the cytoplasm It is due to the effect of the drug on the cell A typical blood picture of this disease is given in the accompanying table and the toxic granulation is illustrated in Figure 7?

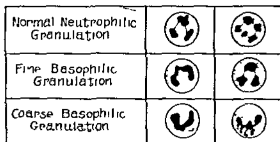



FIG 75 —Basophilic ("toxic") granulation of the neutrophilic leukocytes The toxic granules vary in size depending on the seriousness of the damage to the cells in the marrow (Haden *Principles of Hematology*)

## ERYTHROBLASTOSIS FETALIS

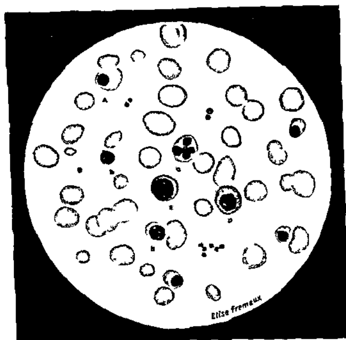
Erythroblastosis fetalis is a disease of the newborn which is associated with a severe hemolytic anemia Before World War II the cause of the disease was unknown and it was often fatal However recent research in this field has uncovered knowledge which promises to salvage untold numbers of erythroblastotic babies The brilliant investigations of Levine Wiener and others indicate that the hemolytic activity is caused by a reaction between agglutinogens and agglutinins

*Table 36 —Typical Blood Picture in Erythroblastosis Fetalis*

| *Significant Examinations* | *Patient s Values* | *Normal Values* |
|---|---|---|
| Red cell count | 1 to 3 million per cu mm | 4 0 to 5 5 million per cu mm |
| Hemoglobin | 3 to 9 Gm | 12 to 17 Gm |
| Blood slide | White cell abnormalities Presence of many metamyelocytes and occasional myelocyte | |
| | Red cell abnormalities Many nucleated red cell mature red cells are large and well filled with hemoglobin | |
| Rh test | Rh positive baby and Rh negative mother | |

The Rh agglutinogens present in the red cells of an Rh positive fetus enter the circulation of the mother and stimulate the production of Rh agglutinins The Rh agglutinins pass into the fetal circulation react with the agglutinogens and cause hemolysis of the cells The treatment in mild cases is to give transfusions with Rh negative blood and in

# PLATE XII




ERYTHROBLASTIC ANEMIA

Wright's stain × 9[illegible]0 *A* Normoblast *B* microblasts *C* normoblast showing karyorrhexis *D* young normoblast showing punctate basophilia *E* lymphocyte (*Whitby, Tice Practice of Medicine courtesy of W F Prior Company*) Note The normoblasts in the above illustration are referred to as rubricytes in the new terminology

severe cases to drain the infant's entire blood supply and simultaneously replace it with compatible blood

The blood picture shows a low hemoglobin and low red cell count The white cell count is usually high and immature cells metamyelocytes and myelocytes appear in the differential One of the most characteristic features of the blood slide is the presence of many nucleated red cells The mature red cells are large and well filled with hemoglobin anisocytosis and poikilocytosis are present Due to the extreme hemolysis of red cells a high icteric index is observed A typical blood picture is given in the accompanying table and the cells of the blood slide are illustrated in Plate XII The normoblasts in the illustration are referred to as rubricytes in the new terminology

## INFECTIOUS MONONUCLEOSIS

(*Synonym* Glandular fever)

Infectious mononucleosis is a mildly contagious disease which is generally *confined to children* and young adults *Its cause is unknown* There are *no specific characteristics of the disease but it* is generally accompanied by soreness of the lymph glands fever nosebleed sore throat and body rashes The disease *usually runs a course of* about 30 days *however, relapses* may occur

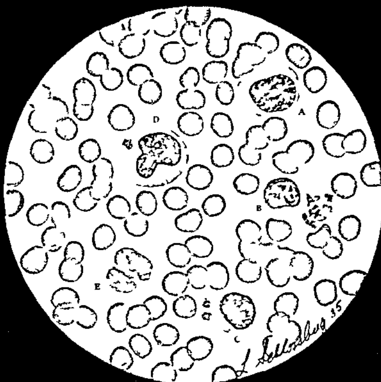
***Table 37 —Typical Blood Picture in Infectious Mononucleosis***

| *Significant Examinations* | *Patient's Values* | *Normal Values* |
|---|---|---|
| Heterophile anti body test | Clumping of cells through 1 112 dilution or higher | Clumping of cells through 1 56 dilution or lower |
| Blood slide | White cell abnormalities 10 to 75% lymphocytes and I M cells | |
| | Red cell abnormalities None | |

Infectious mononucleosis is a difficult disease to diagnose *and the physician relies heavily on the information* furnished by *the technician The blood slide shows* an increase in the number of monocytes and lymphocytes The lymphocytes however *are not the lymphocytes* of normal blood but have a feature which is *characteristic of this disease This feature* is a moth eaten *or vacuolated appearance of the cytoplasm* Several of these cells are *illustrated in Plate XIII*

A typical blood picture in infectious mononucleosis is given in the accompanying table The procedure for the heterophile antibody *test which is mentioned in the table* is given on page 191

# PLATE XIII




INFECTIOUS MONONUCLEOSIS (WRIGHT'S STAIN × 1,200)

*A* Lymphocyte with moderately dense chromatin and vacuolated cytoplasm. *B* small lymphocyte normal except for a few vacuoles in the cytoplasm. *C* lymphocyte with less dense chromatin in the nucleus and several azurophilic granules as well as a few vacuoles in the cytoplasm. *D* lymphocyte which might be mistaken for a monocyte on account of its size and the shape of the nucleus. The coarse character of the chromatin and the blue vacuolated cytoplasm contrast with the fine nuclear chromatin, fine granulation and gray blue cytoplasm of a monocyte *(E)*. (*Wintrobe, Tice Practice of Medicine, courtesy of W. F. Prior Company.*)

# Appendix

Summary of Normal Values

Preparation of Solutions and Reagents

Interpretation of the Terminology

Registry Type Questions and Answers

## Summary of Normal Values

| Test | Normal Values | Common Causes of Increase | Common Causes of Decrease |
|---|---|---|---|
| White cell count | 5,000 to 10,000 per cu. mm. | leukemia, pneumonia, diphtheria, meningitis, and appendicitis | measles, influenza, brucellosis, typhoid fever and malignant neutropenia |
| Red cell count | 4.0 to 5.5 million per cu. mm. | polycythemia vera and dehydration conditions | anemias and leukemias |
| Hemoglobin estimation | 12 to 17 grams per 100 cc. (78 to 110%) | polycythemia vera and dehydration conditions | anemias and leukemias |
| Differential white cell count | Basophilic segmented cells 0 to 1 %<br>Eosinophilic segmented cells 1 to 3 %<br>Neutrophilic band cells 2 to 6 %<br>Monocytes 2 to 6 %<br>Lymphocytes 20 to 35%<br>Neutrophilic segmented cells 55 to 75% | Lymphocytes increased in mumps, whooping cough, infectious mononucleosis and lymphocytic leukemia.<br>Monocytes increased in typhus, brucellosis, tuberculosis and monocytic leukemia.<br>Neutrophilic band and segmented cells increased in acute infections and granulocytic leukemia.<br>Eosinophilic segmented cells increased in asthma, hay fever, scarlet fever, and diseases due to worms. | |

| | | | |
|---|---|---|---|
| Sedimentation rate | *Men* (*mm/hr*) / *Women* (*mm/hr*)<br>Westergren 0 to 9 / 0 to 20<br>Cutler 0 to 8 / 0 to 10<br>Wintrobe-Landberg 0 to 9 / 0 to 20 | acute infection, rheumatic fever, tuberculosis, pregnancy, nephritis and cancer | |
| Hematocrit reading | Men 40 to 50%<br>Women 37 to 47% | dehydration conditions | all types of anemia |
| Sickle cell examination | No sickling present | sickle cell anemia | |
| Fragility test | Beginning of hemolysis 0.44%<br>Completion of hemolysis 0.34% | hemolytic jaundice | obstructive jaundice |
| Reticulocyte count | Adults 1 to 2%<br>Infant 4 to 8% | | some anemias |

# Summary of Normal Values

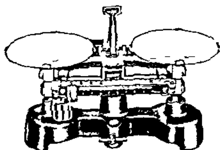
| Test | Normal Values | Common Causes of Increase | Common Causes of Decrease |
| --- | --- | --- | --- |
| White cell count | 5 000 to 10 000 per cu mm | leukemia pneumonia diphtheria meningitis and appendicitis | measles influenza brucellosis typhoid fever and malignant neutropenia |
| Red cell count | 4 0 to 5 5 million per cu mm | polycythemia vera and dehydration conditions | anemias and leukemias |
| Hemoglobin estimation | 12 to 17 grams per 100 cc<br>( 8 to 110%) | polycythemia vera and dehydration conditions | anemias and leukemias |
| Differential white cell count | Basophilic segmented cells 0 to 1 %<br>Eosinophilic segmented cells 1 to 3 %<br>Neutrophilic band cells 2 to 6 %<br>Monocytes 2 to 6 %<br>Lymphocytes 20 to 35%<br>Neutrophilic segmented cells 55 to 75% | Lymphocytes increased in mumps whooping cough infectious mononucleosis and lymphocytic leukemia<br>Monocytes increased in typhus brucellosis tuberculosis and monocytic leukemia<br>Neutrophilic band and segmented cells increased in acute infections and granulocytic leukemia<br>Eosinophilic segmented cells increased in asthma hay fever scarlet fever and diseases due to worms | |

| | | | |
|---|---|---|---|
| L L cells | Nor e found | I L cells pre ent in lupus ery themato us | |
| Eo inophil count | 1 0 to 300 per cu mm | | normally decrea ed after injection of ACTH |
| Spinal cell count | 0 to 10 per cu mm | meningiti encephaliti poliomy eliti and latent yphil s | |
| Heterophile antil ody te t | Pre umptive test clumping of c lls through titer 1 56 or less | infectious mononucleosi erum ickne and after horse serum injections | |
| | Differential te t no clumping of cells | clumping of cells through titer 1 8 or higher in infectious mononucleo is | |

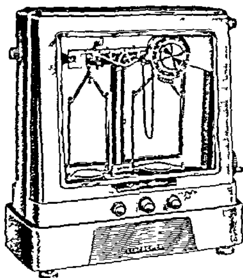
## *Summary of Normal Values (Continued)*

| *Test* | *Normal Values* | *Common Causes of Increase* | *Common Causes of Decrease* |
|---|---|---|---|
| Bleeding time | Duke method 1 to 3 min<br>Ivy method 1 to 3 min | essential thrombocytopenic purpura and hemorrhagic disease of the newborn | |
| Coagulation time | Slide method 2 to 6 min<br>Capillary tube method 2 to 6 min<br>Lee and White method 5 to 10 min<br>Howell method 10 to 30 min | hemophilia obstructive jaundice and hemorrhagic disease of the newborn | |
| Clot retraction time | Some retraction at end of 2 hours and complete retraction at end of 24 hours | essential thrombocytopenic purpura | |
| Prothrombin time | 12 to 17 seconds | dicumarol therapy | |
| Platelet count | 250 000 to 350 000 per cu mm | | essential thrombocytopenic purpura |
| Capillary resistance test | No petechiae formed | petechiae produced in essential thrombocytopenic purpura | |

Since the analytical balance (Fig 76) is much more accurate than the rough balance it is chosen to prepare the majority of solutions and reagents However it is more time consuming and in some cases where accuracy is not



A



B

Fig 76 — Analytical and rough balances. A rough balance B analytical balance

# PREPARATION OF SOLUTIONS AND REAGENTS

This section is the heart of the manual for the accuracy of any determination depends upon the reliability of the reagents If the reagents are off the determination is off regardless of the ability of the technician Consequently it behooves all students to double check the preparation of each solution and reagent and ask when they don t know or are in doubt

### *Table 38 —Commonly Used Weights and Measures*

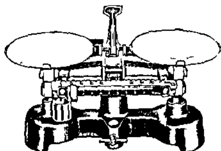
**Weights**

| *Weight* | *Abbreviation* | *Equivalent* | *Rough Example* |
|---|---|---|---|
| milligram | mg | $\frac{1}{1000}$ of a gram | An eyelash weighs about 1 mg |
| gram | Cm | $\frac{1}{1000}$ of a kilogram | A large pea weighs about 1 gram |
| kilogram | kilo | 1000 grams | A kilogram is equal to 2 2 pounds |

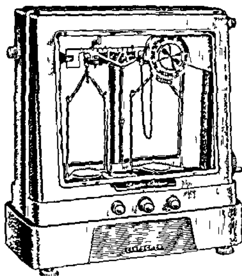
**Measures**

| *Measure* | *Abbreviation* | *Equivalent* | *Rough Example* |
|---|---|---|---|
| cubic centimeter | cc | $\frac{1}{1000}$ of a liter | There are about 15 drops of water in 1 cc |
| milliliter | ml | $\frac{1}{1000}$ of a liter | An ml equals 1 cc Thus there are also about 15 drops of water in 1 ml |
| liter | l | 1000 cc or 1000 ml | A liter is slightly larger than a quart |
| millimeter | mm | $\frac{1}{10}$ of a centimeter | There are about 2.5 cm or 25 mm in 1 inch |
| cubic millimeter | cu mm | $\frac{1}{1000}$ of a cc | In 1 drop of water there are about 6 cu mm. |

Since the analytical balance (Fig 76) is much more accurate than the rough balance it is chosen to prepare the majority of solutions and reagents However it is more time consuming and in some cases where accuracy is not



A



B

Fig 76 —Analytical and rough balances
A rough balance B analytical balance

essential the rough balance may be substituted The type of balance which must be employed is specified under each preparation

There is nothing more distasteful than to see a student (or technician) using the rough balance to weigh out reagents which call for the accuracy of the analytical balance Here they may be allowing a 5 or 10% error to creep into the determination before they even start the test! Not only that think of their coworkers—the poor unenlightened souls who will also use these improperly prepared reagents

The directions for preparing the solutions and reagents are given below The commonly used weights and measures which are used in hematology, are listed in Table 38

**Acetic Acid**

2% acetic acid
Place 98 cc of distilled water in a container
Add 2 cc of glacial acetic acid Mix

**Anticoagulant**

*a* Mixture of ammonium oxalate and potassium oxalate

Using the rough balance weigh out 12 0 grams of ammonium oxalate and 8 0 grams of potassium oxalate Place them in a 1 liter volumetric flask and add distilled water to the 1 liter mark Mix Place 0 5 cc of this solution in a test tube and evaporate to dryness either at room temperature or in an oven which is not over 100° C (The oxalate decomposes at higher temperatures ) The powdered oxalate remaining in the test tube will serve as an anticoagulant for 5 cc of blood

*b* Sequestrene Solution

Using the rough balance weigh out 10 grams of sequestrene Place in a clean bottle and add 100 cc of distilled water Mix When 0 1 cc of this solution is placed in a test tube it will serve as an anticoagulant for 10 cc of blood The solution does not have to be dried it may be used in the liquid form

**Benzidine Solution**

Using the rough balance weigh out 0 1 gram of benzidine Place in 100 cc of distilled water Mix and filter *Just before use* add a few drops of hydrogen peroxide

**Brilliant Cresyl Blue**

a 1% normal saline solution of brilliant cresyl blue

Using the rough balance weigh out 1 gram of brilliant cresyl blue Place in a container and add 99 cc of normal (0 85%) saline Mix and filter

b 1% methyl alcohol solution of brilliant cresyl blue

Using the rough balance weigh out 1 gram of brilliant cresyl blue Place in a container and add 99 cc of methyl alcohol Mix and filter

**Calcium Chloride**

0 025 (fortieth) molar calcium chloride

Using the analytical balance weigh out 1 3875 grams of *anhydrous* calcium chloride Place in a 500 cc volumetric flask and add distilled water to the 500 cc mark Mix

**Copper Sulfate**

0 5% copper sulfate solution

Using the analytical balance weigh out 0 50 gram of *anhydrous* copper sulfate Place in a 100 cc volumetric flask and add distilled water to the 100 cc mark Mix

**Drabkin s Solution** (for cyanmethemoglobin)

This solution is poisonous! Handle with care!

Using the rough balance weigh out 1 0 gram of sodium bicarbonate (C P) Place in a 1 liter volumetric flask

Using the analytical balance weigh out 52 milligrams of potassium cyanide (C P) Add to the volumetric flask.

Using the analytical balance weigh out 198 mg of potassium ferricyanide (C P) Add to the volumetric flask

Add distilled water to the 1 liter mark Mix Transfer to a brown bottle and store in the dark

**Gower s Solution**

Using the analytical balance weigh out 6 250 grams of crystalline sodium sulfate Place in a 100 cc volumetric

flask. Add about 50 cc. of distilled water. Add 16.7 cc. of glacial acetic acid. Dilute to the 100 cc. mark with distilled water. Mix.

### Hayem's Solution

Using the analytical balance, weigh out 0.250 gram of mercuric chloride, 0.50 gram of sodium chloride, and 2.50 grams of crystalline sodium sulfate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

### Hydrochloric Acid

*a.* 1% (approximately 0.1 normal) hydrochloric acid

Place 99 cc. of distilled water in a container. Add 1 cc. of concentrated hydrochloric acid. Mix.

*b.* 0.1 N hydrochloric acid

Using a 10 ml. volumetric pipet, place exactly 10 ml. of 1 N HCl in a 100 ml. volumetric flask. Add distilled water to the 100 ml. mark. Mix.

### Leake and Guy Solution

Using the analytical balance, weigh out 0.050 gram of crystal violet and 1.60 gram of sodium oxalate. Place them in a 100 cc. volumetric flask and add 6 cc. of 40% formaldehyde. Dilute to the 100 cc. mark with distilled water. Heat gently and filter.

### New Methylene Blue N Solution

Using the rough balance, weigh out 0.5 gram of new methylene blue N, 1.4 grams of potassium oxalate, and 0.8 gram of sodium chloride. Place in a clean brown bottle. Add 100 ml. of distilled water. Mix well. Filter before use.

### Normal Saline

*a.* 0.85% (normal) saline solution

Using the analytical balance, weigh out 8.50 grams of sodium chloride. Place in a 1 liter volumetric flask and add distilled water to the 1 liter mark. Mix.

*b.* sterile 0.85% (normal) saline

Using the analytical balance, weigh out 8.50 grams of sodium chloride. Place in a 1 liter volumetric flask

and add distilled water to the 1 liter mark. Mix. Autoclave for 20 minutes at 15 lbs. pressure.

**Oxalated Test Tube**

Using the rough balance, weigh out 12.0 grams of ammonium oxalate and 8.0 grams of potassium oxalate. Place them in a liter volumetric flask and add distilled water to the 1 liter mark. Mix. Place 0.5 cc. of this solution in a test tube and evaporate to dryness either at room temperature or in an oven which is not over 100° C. (Higher temperatures decompose the oxalate.) The powdered oxalate remaining in the test tube will serve as an anticoagulant for 5 cc. of blood.

**Rees and Ecker Solution**

Using the rough balance, weigh out 0.1 gram of brilliant cresyl blue and 3.8 grams of sodium citrate. Place them in a 100 cc. volumetric flask and add 0.2 cc. of 40% formaldehyde. Dilute to the 100 cc. mark with distilled water. Mix.

**Safranin**

1% aqueous solution of safranin.

Using the rough balance, weigh out 1 gram of safranin. Place in a container and add 99 cc. of distilled water. Mix.

**Sequestrene Solution** (*Please look under anticoagulant*)

**Sodium Carbonate**

0.1% sodium carbonate solution.

Using the analytical balance, weigh out 1.0 gram of *anhydrous* sodium carbonate. Place in a 1 liter volumetric flask and add distilled water to the 1 liter mark. Mix.

**Sodium Chloride**

*a.* 0.85% sodium chloride.

Using the analytical balance, weigh out 8.50 grams of sodium chloride. Place in a 1 liter volumetric flask and add distilled water to the 1 liter mark. Mix.

*b.* 0.5% sodium chloride.

Using the analytical balance, weigh out 0.50 gram of sodium chloride. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

**Sodium Citrate**

*a* 3.8% sodium citrate solution

Using the analytical balance weigh out 3.80 grams of sodium citrate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

*b* sterile 3.8% sodium citrate solution

Using the analytical balance weigh out 3.8 grams of sodium citrate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix. Autoclave for 20 minutes at 15 lbs. pressure.

**Sodium Oxalate**

*a* 1.10% sodium oxalate solution

Using the analytical balance weigh out 1.10 grams of *anhydrous* sodium oxalate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

*b* 1.30% sodium oxalate solution

Using the analytical balance weigh out 1.30 grams of *anhydrous* sodium oxalate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

*c* 1.34% sodium oxalate solution

Using the analytical balance weigh out 1.340 grams of anhydrous sodium oxalate. Place in a 100 cc. volumetric flask and add distilled water to the 100 cc. mark. Mix.

**Tannen's Diluting Fluid**

*a* Prepare a 0.2% neutral red iodide solution as follows:

Using the rough balance weigh out 0.5 gram of neutral red iodide. (This reagent may be obtained from your local medical supply house or Allied Chemical and Dye Corp., 40 Rector St., New York, N.Y.) Transfer the 0.5 gram of neutral red iodide to a large bottle. Add 250 cc. of distilled water. Shake for 5 minutes. Filter.

*b* Pour the filtered neutral red iodide into a 100 milliliter volumetric flask until the 100 milliliter mark is reached.

Add exactly 2.1 milliliters of 0.1 N NaOH. Mix well. Filter. Store at room temperature for 6 days and then filter again. This solution is stable.

**Wright's Stain and Buffer Solution**

*a.* Wright's stain

Method one

Using the rough balance, weigh out 3 grams of the powdered Wright's stain. Place in a container and add 1 liter of acetone-free methyl alcohol. Mix. Let stand for several days, mixing a few times each day. Filter before use.

Method Two

Using the rough balance, weigh out 3 grams of powdered Wright's stain. Place in a container and add 30 cc. of glycerol. Mix thoroughly, grinding in a mortar if necessary. Place in a 37° C. incubator or water bath for a few days, stirring a few times each day. With constant stirring, slowly add 1 liter of acetone-free methyl alcohol. Allow to age for a few days. Filter before use. This preparation of Wright's stain is recommended by many technicians.

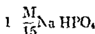
*b.* Buffer solution

Wright's stain contains the acid stain eosin, which is *red in color*, and the basic stain methylene blue, which is blue in color. Consequently, the more acid (lower pH) the buffer solution, the more intense is the red coloration of the cells and the less pronounced is the blue coloration. On the other hand, the less acid (higher pH) the buffer solution, the less intense is the red coloration of the cells and the more pronounced is the blue coloration. Thus it is apparent that the degree of acidity (pH) of the buffer solution is a very important factor in obtaining a good stain.

Experience has shown that a buffer solution which has a pH between 6.4 and 6.8 gives the best results. Some technicians prefer the color contrast which is obtained with a buffer solution of pH 6.4, whereas others

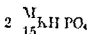
like the buffer of pH 6.8. The best pH value is a matter of individual preference and can be decided by experimentation.

Buffer solutions of various pH values are prepared by mixing two solutions of phosphate salts. The phosphate salt solutions should be kept in pyrex containers at refrigerator temperature. They are prepared as follows:



1. $\frac{M}{15}$ $Na_2HPO_4$

Using the analytical balance, weigh out 9.470 grams of *anhydrous* secondary sodium phosphate $Na_2HPO_4$ (or 11.870 grams of *hydrated* secondary sodium phosphate $Na_2HPO_4 \cdot 2H_2O$). Place in a 1 liter volumetric flask. Add distilled water to the 1 liter mark. Mix.



2. $\frac{M}{15}$ $KH_2PO_4$

Using the analytical balance, weigh out 9.080 grams of primary potassium phosphate $KH_2PO_4$. Place in a 1 liter volumetric flask. Add distilled water to the 1 liter mark. Mix.

To make a buffer solution of the desired pH, mix the quantities of phosphate solutions called for below:

| *pH* | $\frac{M}{15}$ $Na_2HPO_4$ | $\frac{M}{15}$ $KH_2PO_4$ |
|---|---|---|
| 6.4 | 26.7 cc | 73.3 cc |
| 6.5 | 31.8 cc | 68.2 cc |
| 6.6 | 37.5 cc | 62.5 cc |
| 6.7 | 43.5 cc | 56.5 cc |
| 6.8 | 49.6 cc | 50.4 cc |

## INTERPRETATION OF THE TERMINOLOGY

Words in hematology usually consist of one to three parts. They are called stem, prefix, and suffix. For example, the

word hematology is made up of the stem *hemat* meaning blood and the suffix *ology* meaning study. Thus hematology means the study of blood. Erythrocyte consists of the stem *cyte* meaning cell and the prefix *erythro* meaning red. It follows that erythrocyte means red cell. An illustration of a word having all three parts is leukocytosis, which is interpreted as an increase in the number of white cells. It may be broken down as follows:

| *prefix* | *stem* | *suffix* |
|---|---|---|
| leuko | cyt | osis |
| white | cell | increase |

**Table 39.—*Stems, Prefixes and Suffixes Used in the Formation of Hematologic Words***

| *Stem, Prefix or Suffix* | *Meaning* | *Stem, Prefix or Suffix* | *Meaning* |
|---|---|---|---|
| a | without | macro | large |
| aniso | unequal | mega | large |
| baso | base | meniscus | crescent |
| blast | germ | meta | change |
| chrome | color | micro | small |
| crit | separate | mono | one |
| cyte | cell | morpho | form |
| eosin | dawn (red) | myelo | marrow |
| erythro | red | neo | new |
| globin | globe | nucleus | nut |
| granulo | granule | ology | study |
| hem or hemat | blood | penia | poverty |
| hyper | greater | phil | love |
| hypo | less | poiesis | making |
| iso | same | poikilo | variation |
| karyo | nut (nucleus) | poly | many |
| leuk | white | post | after |
| lymph | clear | pro | before |
| lysin | dissolve | reticulo | net |
| | | thrombo | clot |

If the student will keep in mind the meaning of the stems, prefixes, and suffixes given in Table 39, he will have little trouble mastering hematologic terminology. These word parts have their origin in Latin and Greek, since the English language drew heavily upon these sources during its early stages of development.

The list below, arranged in alphabetical order, contains some of the more commonly used words and their definitions. Some words have a more inclusive interpretation than that given, but it is hoped that the restricted definition paints a clearer hematologic picture.

| | |
|---|---|
| **achlorhydria** | Without hydrochloric acid; absence of hydrochloric acid in the gastric juices |
| **agglutinins** | Substances in plasma which may react with agglutinogens and cause clumping or agglutination of the red cells |
| **agglutinogens** | Substances in the red cells which may react with agglutinins and cause clumping or agglutination of the red cells |
| **anisocytosis** | A condition of increased variation in the size of red cells |
| **aplastic** | Lacking any tendency to develop into tissues |
| **band cell** | A cell of the granulocytic series which just precedes the segmented cell |
| **basophilic** | A term describing the granulation found in certain members of the granulocytic series of cells. Basophilic cells have coarse, deep purple granulation |
| **congenital** | Anything, usually an abnormality, which is born with a person |
| **eosinophilic** | A term describing the granulation found in certain members of the granulocytic series. Eosinophilic cells have coarse, red granulation |
| **erythroblastosis** | A condition showing an increased number of immature red cells in the circulating blood |
| **erythrocyte** | The most mature cell of the erythrocytic series |
| **hematocrit** | A centrifuge for separating the cells from the plasma |

| | |
|---|---|
| **hematocrit reading** | The volume per cent occupied by the red cell in centrifuged blood |
| **hematoma** | A black and blue mark caused by the escape of blood from the capillaries or vein into the tissue |
| **hemocytometer** | A counting chamber used to count blood cell |
| **hemoglobin** | A pigment normally found in the red cell which contains iron and is capable of forming a temporary union with oxygen |
| **hemoglobinometer** | An apparatus for measuring hemoglobin |
| **hemophilia** | A hemorrhagic disease characterized by excessive bleeding |
| **hyperchromic** | A term describing red cell which have an excessive amount of hemoglobin (Many authorities state that this term is a misnomer since it is impossible for a red cell to have an excessive amount of hemoglobin) |
| **hypertonic** | A term describing a solution which has a greater concentration (osmotic pressure) than another with which it is associated<br>Example If red cell are placed in a 1 0% salt solution the salt solution is a hypertonic solution because it is more concentrated than the fluid in the cell |
| **hypochromic** | An adjective describing red cells which are deficient in hemoglobin |
| **hypotonic** | A term describing a solution which has a lesser concentration (osmotic pressure) than another with which it is associated<br>Example If red cell are placed in a salt solution of 0 % the salt solution is a hypotonic solution because it has a lesser concentration than the fluid in the cells |
| **idiopathic** | Cause unknown |
| **isotonic** | A term describing a solution which has the same concentration (osmotic pressure) as another solution with which it is associated<br>Example If red cell are placed in a salt solution of 0 8 % the salt solution is isotonic because the salt solution and the fluid in the cells have equal concentrations (osmotic pressure) |

If the student will keep in mind the meaning of the stems prefixes and suffixes given in Table 39 he will have little trouble mastering hematologic terminology. These word parts have their origin in Latin and Greek since the English language drew heavily upon these sources during its early stages of development.

The list below arranged in alphabetical order contains some of the more commonly used words and their definitions. Some words have a more inclusive interpretation than that given, but it is hoped that the restricted definition paints a clearer hematologic picture.

**achlorhydria** Without hydrochloric acid, absence of hydrochloric acid in the gastric juices

**agglutinins** Substances in plasma which may react with agglutinogens and cause clumping or agglutination of the red cells

**agglutinogens** Substances in the red cells which may react with agglutinin and cause clumping or agglutination of the red cells

**anisocytosis** A condition of increased variation in the size of red cells

**aplastic** Lacking any tendency to develop into tissues

**band cell** A cell of the granulocytic series which just precedes the segmented cell

**basophilic** A term describing the granulation found in certain members of the granulocytic series of cells. Basophilic cells have coarse deep purple granulation

**congenital** A anything usually an abnormality which is born with a person

**eosinophilic** A term describing the granulation found in certain members of the granulocytic series. Eosinophilic cells have coarse red granulation

**erythroblastosis** A condition showing an increased number of immature red cells in the circulating blood

**erythrocyte** The most mature cell of the erythrocytic series

**hematocrit** A centrifuge for separating the cells from the plasma

| | |
|---|---|
| poikilocytosis | A condition of increased variation in the shape of red cells |
| polychromasia | Same as polychromatophilia |
| polychromatophilia | A condition of increased variation in the staining qualities of red cells. The cells take a dirty blue to brownish coloration rather than the normal pink color |
| polycythemia | Excessive number of red cells in the blood |
| posthemorrhagic | After hemorrhage |
| progranulocyte | The cell of the granulocytic series which just precedes the myelocyte |
| prolymphocyte | The cell of the lymphocytic series which just precedes the lymphocyte |
| promegakaryocyte | The cell of the thrombocytic series which just precedes the megakaryocyte |
| promonocyte | The cell of the monocytic series which just precedes the monocyte |
| proplasmacyte | The cell of the plasmacytic series which just precedes the plasmacyte |
| prorubricyte | The cell of the erythrocytic series which just precedes the rubricyte |
| prothrombin | A substance which plays an essential role in blood coagulation |
| purpura | A hemorrhagic disease characterized by bleeding into the skin |
| reticulocyte | The cell of the erythrocytic series which just precedes the erythrocyte |
| rouleaux formation | Red cells bunched together like stacks of coins |
| rubriblast | The most immature cell of the erythrocytic series |
| rubricyte | The cell of the erythrocytic series which just precedes the metarubricyte |
| saline | A salt solution |
| segmented cell | The most mature cell of the granulocytic series |
| thrombocyte | The most mature cell of the thrombocytic series |
| thrombocytopenia | Shortage of thrombocytes |
| thromboplastin | A substance found in tissue juices and platelet disintegration which plays an essential part in the coagulation of blood |

| | |
|---|---|
| karyorrhexis | Splitting of nucleus |
| lymphoblast | The most immature cell of the lymphocytic series |
| lymphocyte | The most mature cell of the lymphocytic series |
| macrocyte | A large red cell |
| *megakaryoblast* | *The most immature cell of the thrombocytic series* |
| megakaryocyte | The *cell of the thrombocytic series which* just precedes the thrombocyte |
| metarubricyte | The cell of the erythrocytic series which just precedes the reticulocyte |
| metamyelocyte | The cell of the granulocytic series which just precedes the band cell |
| microcyte | A small red cell |
| monoblast | The most immature cell of the *monocytic* series |
| *monocyte* | The most mature cell of the monocytic *series* |
| myeloblast | The most immature cell of the granulocytic series |
| myelocyte | The cell of the granulocytic series which just precedes the metamyelocyte |
| mononucleosis | An increase in the number of monocytes and lymphocytes |
| *neutrophilic* | A *term describing* the granulation *found in certain* members of the granulocytic series |
| normal saline | A 0.85% *solution of sodium chloride* |
| normochromic | A term describing red cells which are normal in hemoglobin content |
| normocyte | A red cell which is normal *in size* |
| *oxalated test tube* | *Test tube containing an anticoagulant of powdered* ammonium and potassium oxalates |
| *oxyhemoglobin* | Hemoglobin containing oxygen |
| petechia | A small spot formed by the effusion of blood |
| plasmablast | *The most immature cell of the plasmacytic series* |
| plasmacyte | *The most mature cell of the plasmacytic series* |

| | |
|---|---|
| **poikilocytosis** | A condition of increased variation in the shape of red cells |
| **polychromasia** | Same as polychromatophilia |
| **polychromatophilia** | A condition of increased variation in the staining qualities of red cells. The cells take a dirty blue to brownish coloration rather than the normal pink color |
| **polycythemia** | Excessive number of red cells in the blood |
| **posthemorrhagic** | After hemorrhage |
| **progranulocyte** | The cell of the granulocytic series which just precedes the myelocyte |
| **prolymphocyte** | The cell of the lymphocytic series which just precedes the lymphocyte |
| **promegakaryocyte** | The cell of the thrombocytic series which just precedes the megakaryocyte |
| **promonocyte** | The cell of the monocytic series which just precedes the monocyte |
| **proplasmacyte** | The cell of the plasmacytic series which just precedes the plasmacyte |
| **prorubricyte** | The cell of the erythrocytic series which just precedes the rubricyte |
| **prothrombin** | A substance which plays an essential role in blood coagulation |
| **purpura** | A hemorrhagic disease characterized by bleeding into the skin |
| **reticulocyte** | The cell of the erythrocytic series which just precedes the erythrocyte |
| **rouleaux formation** | Red cells bunched together like stacks of coins |
| **rubriblast** | The most immature cell of the erythrocytic series |
| **rubricyte** | The cell of the erythrocytic series which just precedes the metarubricyte |
| **saline** | A salt solution |
| **segmented cell** | The most mature cell of the granulocytic series |
| **thrombocyte** | The most mature cell of the thrombocytic series |
| **thrombocytopenia** | Shortage of thrombocytes |
| **thromboplastin** | A substance found in tissue juices and platelet *disintegration which plays an essential* part in the coagulation of blood |

| | |
|---|---|
| karyorrhexis | Splitting of nucleus |
| lymphoblast | The most immature cell of the lymphocytic series |
| lymphocyte | The most mature cell of the lymphocytic series |
| macrocyte | A large red cell |
| megakaryoblast | The most immature cell of the thrombocytic series |
| megakaryocyte | The cell of the thrombocytic series which just precedes the thrombocyte |
| metarubricyte | The cell of the erythrocytic series which just precedes the reticulocyte |
| metamyelocyte | The cell of the granulocytic series which just precedes the band cell |
| microcyte | A small red cell |
| monoblast | The most immature cell of the monocytic series |
| monocyte | The most mature cell of the monocytic series |
| myeloblast | The most immature cell of the granulocytic series |
| myelocyte | The cell of the granulocytic series which just precedes the metamyelocyte |
| mononucleosis | An increase in the number of monocytes and lymphocytes |
| neutrophilic | A term describing the granulation found in certain members of the granulocytic series |
| normal saline | A 0.85% solution of sodium chloride |
| normochromic | A term describing red cells which are normal in hemoglobin content |
| normocyte | A red cell which is normal in size |
| oxalated test tube | Test tube containing an anticoagulant of powdered ammonium and potassium oxalates |
| oxyhemoglobin | Hemoglobin containing oxygen |
| petechia | A small spot formed by the effusion of blood |
| plasmablast | The most immature cell of the plasmacytic series |
| plasmacyte | The most mature cell of the plasmacytic series |

*b* dry heating in an oven at 100 C for 1 hr
*c* boiling in water for 30 min and drying in an oven

4 Which of the following blood preparations is not a liquid pages 27-31
*a* blood serum
*b* blood smear
*c* blood plasma
*d* cell suspension

5 Which of the following substances is not used in the coagulation of blood pages 28 132
*a* prothrombin
*b* calcium
*c* sodium chloride
*d* fibrinogen

6 Which of the following is not an anticoagulant page 29
*a* heparin
*b* potassium oxalate
*c* calcium chloride
*d* sequestrene

7 The normal values for the white cell count are page 33
*a* 4 000 to 8 000 per cu mm
*b* 8 000 to 12 000
*c* 5 000 to 10 000

8 A CBC does not include the following test page 23
*a* white cell count
*b* platelet count
*c* differential white cell count
*d* red cell count

9 Which of the following solutions is not used as a diluting fluid for the white cell count page 35

# REGISTRY TYPE QUESTIONS AND ANSWERS

This section contains 106 type questions found on Registry examinations

The first part of the section contains 66 multiple choice questions The second part contains 40 true-false type questions The answers to all questions are given at the end of the section As an added convenience to the student the pages of reference are listed with each question

Some problems call for calculations which the average person can not make "in his head" For these calculations the student should use scratch paper

Place a circle or bracket around the letter which you consider the best answer to the question or problem

Example

The word leukocytosis refers to a

- a Low white cell count
- [b] High white cell count
- c Normal white cell count

| *Question or Problem* | *Where Discussed* |
|---|---|
| 1 The average man has approximately the following volume of blood<br>a 3 quarts (about 3 liters)<br>b 5 quarts<br>c 7 quarts | page 10 |
| 2 Which of the following needles has the bore with the widest diameter<br>a 19 gauge<br>b 20 gauge<br>c 21 gauge | page 16 |
| 3 Which method is *not* satisfactory for sterilizing needles and syringes<br>a autoclaving for 20 min at 15 lbs pressure | page 18 |

number in 1 0 cu mm the volume correction factor is

a 50
b 100
c 200
d 10

15 Hemoglobin normally occurs in the circulating blood as reduced hemoglobin and page 56

a sulfhemoglobin
b oxyhemoglobin
c methemoglobin

16 The normal values for hemoglobin are page 56

a 6 to 10 grams per 100 cc
b 10 to 14
c 12 to 17
d 4 to 8 micrograms per 100 cc

17 Which of the following does not belong to the white cell series of cells page 75

a monocytic series
b granulocytic series
c erythrocytic series
d plasmacytic series

18 In a normal differential white cell count you would expect to find the following per cent of neutrophilic segmented cells page 77

a 20 to 35%
b 2 to 6 %
c 55 to 75%
d 70 to 90%

19 The buffer solution used with Wright s stain should have the following pH page 83

a 6 4 to 6 8
b 6 0 to 6 4
c 6 8 to 7 2

a 1% hydrochloric acid
b 2% acetic acid
c 1% sodium chloride ✓

10 *The counting chamber has a depth of 0 1 mm The white cells are counted in the four large corner squares each square having an area of 1 sq mm The volume correction factor for the white cell count is* page 46

a 5
b 2.5
c 50
d 20

11 *Which solution is not used as a diluting fluid for the red cell count* page 49
a Gower s solution
b Rees and Ecker solution
c Hayem s solution

12 *Since the blood is diluted 0 5 to 100 in the red cell count the dilution correction factor is* page 53

a 100
b 200
c 20

13 In the Spencer bright line hemocytometer the chamber depth is 0 1 *mm* *What is the volume over 16 of the smallest squares* (1 R section) *in the ruled area* page 51

a 0.004 cu mm
b 0.010 cu mm
c 0.025 cu mm
d 0.020 cu mm

14 In the red cell count the cells are counted *in* 0.02 *cu mm of solution Since the report is to be given as the* page 53

*b* Tallqvist
*c* Westergren
*d* Wintrobe Landsberg

26 Tilting the tube used in sedimentation rate determinations will cause the rate to be — page 94

*a* unchanged
*b* decreased
*c* increased

27 The normal values for the hematocrit reading in men are — page 102

*a* 0 to 9 mm per hour
*b* 0 to 20 mm per hour
*c* 30 to 40%
*d* 40 to 50%

28 Which of the following is not a method for determining the hematocrit reading — page 103

*a* Wintrobe
*b* Van Allen
*c* Peters and Van Slyke
*d* Sanford Magath

29 The red cell count is normal and the cells of the blood slide appear to be normochromic and normocytic the hematocrit reading will be close to — page 102

*a* 70%
*b* 40%
*c* 100%
*d* 9 mm per hour

30 In the red cell fragility test the cells of a normal person begin to hemolize at a salt concentration of — page 112

*a* 0 50%
*b* 0 44%
*c* 0 34%
*d* 0 30%

20 An eosinophilic band cell is a member of the following series of cells Plate II page 76

- *a* lymphocytic
- *b* monocytic
- *c* granulocytic
- *d* plasmacytic

21 A white cell count is below normal and the differential shows an increased number of band cells. According to the Schilling system it would be considered a page 89

- *a* regenerative shift to the left
- *b* shift to the right
- *c* degenerative shift to the left

22 Variation in the size of red cells is known as page 92

- *a* poikilocytosis
- *b* polychromatophilia
- *c* basophilic stippling
- *d* anisocytosis

23 A red cell has a clear rim around the edges with an area of hemoglobin concentration in the middle. It is page 92

- *a* hyperchromic
- *b* anisocytic
- *c* a target cell
- *d* a basket cell

24 The immediate precursor of the erythrocyte is the Plate IV page 92

- *a* normoblast
- *b* megaloblast
- *c* reticulocyte
- *d* metarubricyte

25 Which of the following is not a method for determining the sedimentation rate page 94

- *a* Cutler

*b* Tallqvist
*c* Westergren
*d* Wintrobe-Landsberg

26. Tilting the tube used in sedimentation rate determinations will cause the rate to be — page 94
   *a* unchanged
   *b* decreased
   *c* increased

27. The normal values for the hematocrit reading in men are — page 102
   *a* 0 to 9 mm per hour
   *b* 0 to 20 mm per hour
   *c* 30 to 40%
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   *a* Wintrobe
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   *a* 70%
   *b* 40%
   *c* 100%
   *d* 9 mm per hour

30. In the red cell fragility test the cells of a normal person begin to hemolize at a salt concentration of — page 112
   *a* 0.50%
   *b* 0.44%
   *c* 0.34%
   *d* 0.30%

20 An eosinophilic band cell is a member of the following series of cells — Plate II, page 76

- *a* lymphocytic
- *b* monocytic
- *c* granulocytic
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- *a* regenerative shift to the left
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- *a* normoblast
- *b* megaloblast
- *c* reticulocyte
- *d* metarubricyte

25 Which of the following is not a method for determining the sedimentation rate — page 94

- *a* Cutler

*b* 1 to 5 mm
*c* 3 to 5 mm
*d* 6 to 10 mm

37 Which of the following is not a method for the coagulation time — page 135
*a* Wintrobe method
*b* Capillary tube method
*c* Lee and White method
*d* Howell method

38 The normal values for the Lee and White coagulation time are — page 135
*a* 10 to 30 min
*b* 1 to 3 min
*c* 2 to 6 min
*d* 5 to 10 min

39 The normal values for the prothrombin time are — page 140
*a* 1 to 3 min
*b* 5 to 10 min
*c* 12 to 17 sec
*d* 20 to 25 sec

40 During treatment with dicumarol the prothrombin time is — page 140
*a* normal
*b* increased
*c* decreased

41 The normal values for the platelet count are — page 146
*a* 5 000 to 1 000 cell per cu mm
*b* 1 to 2%
*c* 250 000 to 350 000 cells per cu mm

42 Which of the following is not a method for the platelet count — page 147
*a* direct method
*b* Ivy method
*c* indirect method

31 In adults the normal values for the reticulocyte count are page 118

*a* 8 to 10%
*b* 1 to 2 %
*c* 6 to 8 %
*d* 4 to 6 %

32 Which of the following is used for staining reticulocytes page 117

*a* gentian violet
*b* brilliant green
*c* brilliant cresyl blue
*d* Wright's

33 The normal value for the hemoglobin is given as *14.5 grams per 100 cc* and the normal value for the RBC is given as 5 million per cu mm If the patient's hb is 11.0 grams and his RBC is 4 million the color index is page 124

*a* 0.85
*b* 0.95
*c* 1.10
*d* 0.98

34 The mean corpuscular volume of a healthy person is page 125

*a* 50 to 60 cu microns
*b* 80 to 90 cu microns
*c* 95 to 100 cu microns

35 In the formation of a blood clot fibrinogen is transformed to page 132

*a* thrombin
*b* thromboplastin
*c* prothrombin
*d* fibrin

36 The normal values for the bleeding time are page 133

*a* 4 to 8 min

49 Which of the following is not needed in a cross match page 167

- *a* patient's serum
- *b* 0.5% sodium chloride
- *c* donor's serum
- *d* patient's cell suspension

50 In the direct Coombs test the following blood preparation is required page 174

- *a* cell suspension from father
- *b* cell suspension from infant
- *c* serum from mother

51 An offspring with erythroblastosis fetalis commonly results from an page 176

- *a* Rh positive mother and Rh negative father
- *b* Rh negative mother and Rh positive father
- *c* Rh positive mother and Rh positive father

52 Which reagent or preparation is not used in an Rh titer page 176

- *a* normal (0.85%) saline
- *b* bovine albumin
- *c* fresh group O Rh positive blood
- *d* sheep cells

53 The I I cell may be found in the following disease page 179

- *a* leukemia
- *b* lupus erythematosus
- *c* infectious mononucleosis
- *d* hemophilia

54 The normal values for the eosinophil count are page 181

- *a* 10 to 15%
- *b* 150 to 350 cells per cu mm
- *c* 1 to 4

1 *a*

43 The clot retraction time is related to — page 139

*a* white cell count
*b* platelet count
*c* coagulation time
*d* prothrombin time

44 Which of the following is used in the capillary resistance test — page 152

*a* Gram's iodine
*b* Leeke and Guy solution
*c* blood pressure apparatus
*d* normal (0.85%) saline

45 People in blood group A have the following agglutinin in their plasma — pages 154-155

*a* a
*b* none
*c* b

46 Group B blood contains the following agglutinogens in the red cells — page 154

*a* A
*b* B
*c* O

47 Which of the following blood groups is called the universal donor — page 156

*a* B
*b* O
*c* A
*d* AB

48 In the slide method of ABO grouping if the blood is clumped by group A (anti-B) serum and also by group B (anti-A) serum it belongs to group — pages 161-162

*a* A
*b* B
*c* AB
*d* O

61 The stain used to differentiate immature members of the granulocytic and lymphocytic series is known as page 211

- *a* Wright's stain
- *b* cresyl blue
- *c* peroxidase stain
- *d* Giemsa stain

62 In doing a differential white cell count on a patient with chronic granulocytic leukemia you would expect to find from 5 to 35% page 213

- *a* myeloblasts
- *b* myelocytes
- *c* prorubricytes
- *d* rubriblasts

63 Which test is not increased in essential thrombocytopenic purpura page 218

- *a* bleeding time
- *b* platelet count
- *c* clot retraction time

64 Which test is not increased in hemorrhagic disease of the newborn page 222

- *a* coagulation time
- *b* bleeding time
- *c* clot retraction time
- *d* prothrombin time

65 In polycythemia vera you would expect to find page 224

- *a* normal red cell count
- *b* low hemoglobin
- *c* low hematocrit reading
- *d* high red cell count

66 In malignant neutropenia you would expect to find page 226

- *a* high white cell count
- *b* normal white cell count

55 The heterophile antibody test is a useful diagnostic aid in — page 191

- *a* malignant neutropenia
- *b* infectious mononucleosis
- *c* leukemia
- *d* pernicious anemia

56 Which reagent is not used in the heterophile antibody test — pages 191 194

- *a* 0 5% sodium chloride
- *b* suspension of sheep cells
- *c* 0 85% sodium chloride
- *d* guinea pig kidney antigen

57 Sickle cell anemia is a — page 197

- *a* deficiency anemia
- *b* aplastic anemia
- *c* hemolytic anemia
- *d* hemorrhagic anemia

58 In aplastic anemia the reticulocyte count is — page 202

- *a* normal
- *b* increased
- *c* decreased

59 The blood picture during a relapse of congenital hemolytic anemia shows — page 206

- *a* sickle cells
- *b* spherocytes
- *c* target cell
- *d* megaloblasts

60 Which of the following is not classified as a leukemia — page 210

- *a* granulocytic leukemia
- *b* plasmacytic leukemia
- *c* normocytic leukemia
- *d* monocytic leukemia

| | | | | |
|---|---|---|---|---|
| 8 | When blood is drawn to the 0.5 mark and diluting fluid to the 101 mark of the red cell pipet the blood is diluted 1 in 200 | T | F | page 50 |
| 9 | An eosinophilic leukocytosis is usually found in asthma hay fever and parasitic infestations | T | F | page 77 |
| 10 | Wright s stain is a methyl alcohol solution of eosin and gentian violet | T | F | page 82 |
| 11 | The sedimentation rate is increased in acute infections rheumatic fever pregnancy and cancer | T | F | page 94 |
| 12 | In the Wintrobe-Landsberg method for the sedimentation rate the normal values for men are 0 to 20 mm per hour | T | F | page 94 |
| 13 | In dehydration conditions such as a severe burn case you would expect to find a low hematocrit reading | T | F | page 102 |
| 14 | If the oxygen is removed from the red cells of a person with sickle cell anemia the red cells become sickle shaped | T | F | page 107 |
| 15 | In hemolytic jaundice the fragility of of the red cells is decreased | T | F | page 109 |
| 16 | In the fragility test the red cells are placed in a hypertonic salt solution | T | F | page 109 |
| 17 | The mean corpuscular hemoglobin is the weight of hemoglobin in the average red cell | T | F | page 121 |
| 18 | In determinations of the coagulation time the capillary blood methods have a shorter coagulation time than the venous blood methods | T | F | page 135 |

c high hematocrit reading
d low white cell count

The following statements are either true or false If the statement is true place a bracket or circle around the T if it is false place a bracket or circle around the F

*Example*

| | | |
|---|---|---|
| A promyelocyte is an immature white cell | [T] F | |
| 1 One of the functions of the blood is to aid in regulating the water content temperature and alkalinity of the tissues | T F | page 9 |
| 2 For every white cell there are approximately 400 platelets and 1000 red cells | T F | page 10 |
| 3 In making a venipuncture the tourniquet is released after the needle is is withdrawn from the vein | T F | pages 19 21 |
| 4 The needle should be removed from the syringe before the blood is expelled into a test tube | T F | page 26 |
| 5 Serum differs from plasma in that it contains no fibrinogen | T F | page 31 |
| 6 When the red cells are placed in a hypotonic salt solution water enters the cells and causes them to swell | T F | page 31 |
| 7 When blood is drawn to the 0 5 mark and diluting fluid to the 11 0 mark of the white cell pipet the blood is diluted 1 in 20 | T F | page 37 |

30 Since the cells in spinal fluid disintegrate rapidly, the spinal count should be made while the fluid is fresh T F page 184

31 Spinal fluid often contains contagious material T F page 184

32 The best time to obtain blood for a malaria smear is during the chills and fever stage T F page 187

33 Bone marrow smears are of particular interest in aplastic anemia, pernicious anemia, and multiple myeloma T F page 189

34 Pernicious anemia is one of the deficiency anemias T F page 197

35 In simple achlorhydric anemia the red cells of the blood slide are unusually large and well packed with hemoglobin T F page 200

36 Hypochromic microcytes may be found in the blood slide during acute posthemorrhagic anemia T F page 208

37 A typical blood picture in chronic lymphocytic leukemia shows a white cell count of 40,000 to 80,000 cells per cu mm and a differential of 80 to 95% lymphocyte T F page 214

38 In hemophilia the bleeding time is usually normal but the coagulation time is increased T F page 220

39 In erythroblastosis fetalis one of the most characteristic features of the blood slide is the presence of many nucleated red cells T F page 229

40 You would expect to find many abnormal red cells but no abnormal white cells in infectious mononucleosis T F page 230

| | | |
|---|---|---|
| 19 In dicumarol therapy a hemorrhage may occur if the clotting activity of the blood is greatly decreased | T F | page 140 |
| 20 In prothrombin time determinations if the plasma is not used immediately it should be stored in the refrigerator | T F | pages 141 143 |
| 21 When platelets disintegrate they release thromboplastin one of the essential factors in coagulation | T F | page 146 |
| 22 The capillary resistance test measures the ability of the capillaries to resist pressure | T F | page 150 |
| 23 In the transfusion of blood the red cell agglutinogens entering the patient must not meet similar agglutinins | T F | pages 155 156 |
| 24 About 45% of the population is in blood group O | T F | page 154 |
| 25 Group O blood has no agglutinins in its plasma | T F | pages 154 155 |
| 26 Rh agglutinins may be produced by an Rh positive person receiving Rh negative blood | T F | pages 157 158 |
| 27 In the slide method for Rh typing Rh positive blood usually takes from 5 to 10 minutes to clump | T F | page 160 |
| 28 In a cross match the major side determines the compatibility of the donor's serum and the patient's cells | T F | page 166 |
| 29 If the mother is Rh negative and the father is Rh positive there is a possibility of having an erythroblastotic baby | T F | page 170 |

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*Answers to Registry Type Questions*

*Multiple Choice Questions*

| | | | | | |
|---|---|---|---|---|---|
| 1 | b | 23 | c | 45 | c |
| 2 | a | 24 | c | 46 | b |
| 3 | b | 25 | b | 47 | b |
| 4 | [illegible] | 26 | c | 48 | c |
| 5 | c | 27 | d | 49 | [illegible] |
| 6 | c | 28 | c | 50 | b |
| 7 | c | 29 | b | 51 | b |
| 8 | b | 30 | b | 52 | d |
| 9 | [illegible] | 31 | b | 53 | [illegible] |
| 10 | b | 32 | c | 54 | b |
| 11 | b | 33 | b | 55 | [illegible] |
| 12 | b | 34 | [illegible] | 56 | a |
| 13 | a | 35 | d | 57 | c |
| 14 | a | 36 | b | 58 | c |
| 15 | [illegible] | 37 | a | 59 | b |
| 16 | c | 38 | d | 60 | |
| 17 | c | 39 | c | 61 | c |
| 18 | | 40 | b | 62 | b |
| 19 | a | 41 | c | 63 | [illegible] |
| 20 | c | 42 | b | 64 | c |
| 21 | c | 43 | b | 65 | [illegible] |
| 22 | [illegible] | 44 | c | 66 | d |

*True False Questions*

| | | | |
|---|---|---|---|
| 1 | T | 21 | T |
| 2 | F | 22 | T |
| 3 | F | 23 | T |
| 4 | T | 24 | T |
| 5 | T | 25 | F |
| 6 | T | 26 | F |
| 7 | T | 27 | F |
| 8 | T | 28 | F |
| 9 | T | 29 | T |
| 10 | F | 30 | T |
| 11 | T | 31 | T |
| 12 | F | 32 | T |
| 13 | F | 33 | T |
| 14 | T | 34 | T |
| 15 | F | 35 | F |
| 16 | F | 36 | T |
| 17 | T | 37 | T |
| 18 | T | 38 | T |
| 19 | T | 39 | T |
| 20 | T | 40 | F |

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